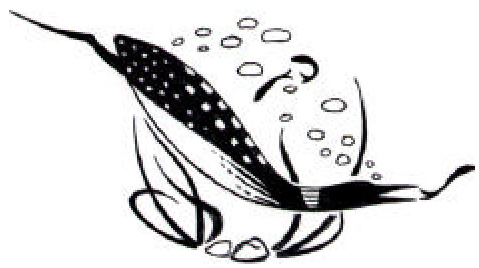
Assessing the impacts of methylmercury on piscivorous wildlife: as indicated by the Common Loon, 1998-2000
(Report BRI00-01)



2000 Final Report

Submitted to:

Maine Department of Environmental Protection
Surface Water Ambient Toxic Monitoring Program
State House Station 17
Augusta, Maine 04333

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23 March 2001

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Executive Summary:

Anthropogenic inputs of mercury (Hg) into the environment have significantly increased in the past few decades. In conjunction, the current availability of methylmercury (MeHg) in aquatic systems has increased to levels posing risks to human and ecological health. Risk levels vary considerably in response to MeHg availability, which is affected by lake hydrology, biogeochemistry, habitat, topography, and proximity to airborne sources. We selected the Common Loon as the most suitable bioindicator of aquatic Hg toxicity, based on ecological, logistical, and other criteria, including public valuations of natural resources. Opportunistic and probability-based sampling efforts from 1994-2000 indicate New England's breeding loon population is at unacceptable levels of risk to Hg contamination, particularly in Maine. Based on risk categories developed from the literature and *in situ* studies by BioDiversity Research Institute and their collaborators, 30% of the breeding loon population in Maine is estimated to be at risk, while 46% of the eggs laid are potentially impacted.

Because results from national sampling indicated loons were at most risk from Hg in New England (particularly Maine), we identified several individual- and population-level parameters to better understand the extent of mercury toxicity across Maine. Between 1994-00, we collected 139 abandoned eggs as well as blood and feather samples from 253 adult and 103 juvenile wild loons captured in Maine. The Hg concentrations in these samples were used to characterize sublethal impacts of Hg on egg development, behavior, developmental stability, immunosuppression, individual survival, and overall reproductive success. In the Rangeley Lakes Study Area, a total of 185 loon territories were monitored on 43 lakes during 1998-00. Current monitoring efforts and historical data comprise 515 territory-years measured. Behavioral observations were conducted for over 1,500 hours on 16 lakes with 38 loon territories from 1998 to 2000.

Several reproductive measures significantly declined for loon pairs at high risk to prey MeHg availability, thereby corroborating studies in high-risk sites in Nova Scotia and Wisconsin that show Hg impacts reproductive success. Based on 223 loon territories representing 748 territory-years surveyed we found that extra-high risk pairs fledged 37% fewer young than pairs at low risk to Hg. We also found similar significant patterns of lower productivity on high and extra-high risk territories compared to low and moderate risk territories for other reproductive measures. We view the implication of long-term declines in these reproductive measures are serious and contend they would not be detected by traditional survey techniques.

Insight into why loons are facing Hg-based population declines can be seen through our hazard assessment process that is based on a weight of evidence approach. Physiological impacts of Hg are measured through two key biomarkers: corticosterone stress hormone levels and flight feather asymmetry. Circulating corticosterone hormone levels are strongly linked with increasing blood Hg levels and are not related to capture and handling stress. Corticosterone hormone levels increase on an average of 14.6% for every one ppm of increase in blood Hg levels (n=239). This indicates that loons with high blood Hg levels have higher rates of chronic stress and may therefore have compromised immune systems. Asymmetry measurements provide insights into developmental stability and potentially reproductive fitness. Three years of flight feather measurements have shown annual agreement that loon breeding populations with greater exposure to Hg have significantly greater asymmetry than populations at low risk (n=227). Greater asymmetry may indicate disruptions from stressors on their embryonic development and current physiological status as well as a potential decline in reproductive fitness.

Many behavioral impacts that appear to be related to the neurotoxic effects of MeHg can rarely be observed in the field. We found adult loons in high risk situations left eggs uncovered 14% of the time, compared to 1% in controls. Several cases of direct field observations indicate that adult loons with high Hg body burdens avoid incubating their eggs and display atypical behaviors such as patrolling in front of, or sitting

next to the nest. We documented a significant negative relationship between adult blood Hg and foraging behavior, and a significant positive relationship between adult blood Hg and brooding behavior. Recategorizing our data according to energy demands revealed a significant inverse relationship between blood Hg and time spent in high energy behaviors. Our findings are consistent with other studies linking Hg and lethargy, reduced motivation to hunt prey, and compromised foraging abilities.

Current levels of Hg in Maine's lacustrine ecosystems also appear to be impacting individual survival of adult and juvenile loons. Recaptured adult loons exhibit a significant annual increase of Hg (9% in males, 5.6% in females) that we predict will significantly reduce lifetime individual performance. A model of this impact indicates a decline of 13 to 8 young produced over a loon's lifetime. Further, juveniles from high-risk territories have significantly increasing blood Hg levels of 3% per day during the summer, potentially reaching dangerous levels after the final feather molt at 11 weeks of age.

Characterization of the risk imposed by MeHg bioavailability in aquatic systems to high trophic level obligate piscivores such as the Common Loon indicates negative population level impacts in Maine. Although the impacts of Hg on loons are varied, complex, and not yet fully understood, the combination of high exposure to a significant part of the breeding population and the "bottom-line" impact of reducing overall reproductive success to 37%, has created an aquatic landscape that is not sustainable for the Common Loon in Maine.

Current models indicate a negative population growth rate. Because of the loon's life history strategy (i.e., long lived, slow maturing, and low fecundity) the annual and continual impacts of this type of stressor causes an erosion of the non-breeding or buffer population that serves as a natural cushion to catastrophic events. Once this buffer population is exhausted, the occupancy of established territories will shrink and it will be more obvious that loon populations are declining. However, the realization of shrinking loon populations at that stage will require drastic and potentially expensive efforts to reverse the decline. Models based on a 25-year, statewide comprehensive monitoring effort in New Hampshire show approximately half of Maine's buffer population has been exhausted. Certain areas in Maine, such as the Allagash area that may be particularly impacted from Hg, may already exhibit exhaustion of the buffer population and a shrinking number of territorial pairs.

Continued refinement of model parameters and either a probability-based sampling scheme or new sampling efforts in northern Maine will provide higher confidence in our estimates that will therefore assist in state-based policy efforts as well as national regulations that reflect the ecological injury Hg is currently having on the freshwater landscape.

Introduction

Due to high concentrations of mercury (Hg) in fish from Maine lakes, ponds, rivers, and streams, the Maine Bureau of Health issued a statewide "fish consumption advisory" in 1994, (modified in 1997) warning Maine citizens to limit consumption of fish from all fresh waters. Impacts on wildlife, however, are less well known in Maine and elsewhere. Recent ecological concerns were highlighted at the "New England Governors and Eastern Canadian Premiers" conference sponsored by the USEPA and Maine Department of Environmental Protection. Recommendations from the report 'Northeast States and Eastern Canadian Provinces Mercury Study: A Framework for Action' stated: "conduct additional research on the cycling and bioavailability of mercury in aquatic ecosystems and on the ecological impacts of elevated fish mercury levels, particularly for fish-eating wildlife such as eagles, loons, osprey, otter, and mink" (NESCAUM 1998).

In addition, strategy 9 from "Mercury in Maine," a report by the Land and Water Resources Council to the Maine legislature in January 1998, recommends "focus biological research efforts on the effects of mercury on the health of loons, fish and other wildlife with elevated mercury levels." Emphasis from policy makers and researchers has been on higher trophic level piscivorous wildlife since they are most at risk due to mercury's ability to bioaccumulate and biomagnify (Scheuhammer 1991, Thompson 1996, U.S. EPA 1997). This interest has facilitated a new initiative by the USEPA-Office of Research and Development's NHEERL program to investigate stressors (such as Hg) using a focal species (such as the loon) to provide geographically relevant empirical information for science-based policy.

Using Birds as Bioindicators of MeHg Availability

The use of piscivorous birds as indicators of MeHg availability is common (e.g., Thompson 1996, Evers et al. 1998a,b, Wolfe 1998, Wolfe and Norman 1998). We believe piscivorous birds are also useful as general ecological indicators of aquatic ecosystem integrity and of the presence and effects of environmental stressors.

Mercury deposition and MeHg availability is now sufficiently elevated in the Northeast region to cause impacts on wildlife (Welch 1994, Burgess et al. 1998, Nocera and Taylor 1998). Based on the USEPA probability-based sampling efforts in the USEPA's Region 1 and 2, Yeardley et al. (1998) predicted that 98% of New England's lakes contained fish with MeHg levels exceeding critical values for piscivorous birds. In corroboration, Evers et al. (1998a) found Common Loons (*Gavia immer*) breeding in Region 1 (MA, ME, NH, RI, VT) had the highest mean blood Hg levels in the United States, while juvenile loon blood Hg levels were four times those of the designated reference site in Alaska. Further studies on a suite of five piscivorous birds in Maine indicated over 70% of lakes have the capacity to produce MeHg at levels above designated risk categories (Evers et al. 1998b). These studies demonstrate that extensive Hg contamination and MeHg availability exists in Region 1.

Yeardley et al. (1998) found from analyzing 11 metals in fish throughout the U.S. that, "MeHg was determined to be the elemental contaminant of regional concern to fish consumers." Our study focused on assessing the ecological risk of Hg to a piscivorous bird—the Common Loon. We selected the loon as our bioindicator because of a vast amount of information available on its demographics (e.g., Piper et al. 1997a, Piper et al. 1997b, Evers et al. 2000, Evers 2001), behavioral ecology (e.g., Evers 1993, Nocera and Taylor 1998, Paruk 1999), toxicology (e.g., Evers et al. 1998a, b, Meyer et al. 1998, Scheuhammer et al. 1998a, b), and local breeding population status (Maine Audubon Society and BioDiversity Research Institute unpubl. data).

Mercury Risk to Loons

An estimated 21-37% of the New England Common Loon breeding population has Hg levels that exceed wildlife safety thresholds designated by other studies (e.g., Barr 1986, Scheuhammer 1991, Thompson 1996, Burgess et al. 1998, Meyer et al. 1998). In addition, over 60% of abandoned loon eggs collected in Region 1 (n=305) have Hg levels considered elevated (i.e., 0.5 ppm) by laboratory studies (Fimreite 1971, Heinz 1979) and 5% have lethal levels (i.e., 2.0 ppm) (Thompson 1996).

These and previous studies documenting exposure in Maine loons (Evers and Reaman 1998, Evers et al. 1998b) predict that impacts are likely. This study was initiated to (1) determine the extent of actual impacts on loons in Maine's lakes and ponds and (2) improve confidence levels on the impact thresholds used to establish risk. We and other collaborators believe relationships exist between high Hg levels and: (1) decreased egg laying capability, (2) decreased egg hatchability, (3) altered parental investment, (4) altered chick behavior, (5) reduced fitness in adults and juveniles, (6) decreased juvenile survival, and (7) reduced lifetime reproductive success. Complementary collaborative studies in the Great Lakes, other New England states, and Canadian Provinces aid in interpretation of our results.

Study Area

BioDiversity Research Institute (BRI) has collected exposure, demographic, and physiological information on New England's breeding loon populations since 1993. Much of this research has been based in the upper Androscoggin and Kennebec River watersheds (e.g., Evers and Reaman 1998, Evers et al. 1998a,b, Evers et al. 1999, Evers 2001). Because of this knowledge base and some of the highest Hg levels recorded for Common Loons in North America, we have continued our focus on the Rangeley Lakes area for a high resolution study on the potential impacts of Hg to wildlife (Figure 1). Besides the 43 study lakes and 185 territories in the Rangeley Lakes Study area (including Lake Umbagog), 38 other territories from outside this region were used to further develop insight into population level impacts of Hg.

Lakes were classified as natural or those impounded by dams (i.e., reservoirs). We monitored 128 loon territories on seven reservoirs and 57 loon territories on natural lakes (Table 1). Impoundments were defined according to their water management regime by Evers and Reaman (1998): Regulated storage reservoir (RSR) had annual fluctuations greater than 1.5 m, regulated peak reservoirs (RPR) had weekly water fluctuations over 1 m, while regulated full ponds (RFP) were raised lakes managed for minimal water level fluctuations of less than 1.5 m. Drainage lakes had both an inlet and outlet where the primary water source is stream drainage while spring lakes have no inlet but do have an outlet. The water source for spring lakes is the groundwater flow from the immediate drainage area.

Because water level fluctuations from reservoirs impact loon reproductive success more than natural lakes, we only included territorial pairs using rafts floated as part of FERC relicensing. BRI staff monitor loon territories on Flagstaff and Aziscohos Lakes as part of a license agreement by FPL Energy Maine Hydro. Most territories on Aziscohos, Flagstaff, and Wyman have rafts. There are no rafts on Mooselookmeguntic and Richardson and water level changes on Rangeley and Umbagog are minimal.

Table 1. Common Loon territories monitored for overall reproductive success and sampled for Hg exposure.

Lake	Lake Type	# of territory-years used	# of territories monitored	# of territories w/ Hg levels	Low Hg	Mod. Hg	High Hg	Xhigh Hg
Arnold	natural	1	1	0	0	0	0	0
Aziscohos	Reservoir-RSR	186	23	15	0	6	5	4
B Pond	natural	1	1	0	0	0	0	0
Beaver Mountain	natural	1	1	1	1	0	0	0
Big Beaver	natural	1	2	0	0	0	0	0
Big Jim	drainage	7	3	3	3	0	0	0
C Pond	natural	1	1	0	0	0	0	0
Chain-of-Ponds	drainage	4	3	2	0	1	0	1
Cranberry	natural	1	1	1	1	0	0	0
Crosby	natural	1	1	1	0	1	0	0
Dodge	drainage	1	1	0	0	0	0	0
East Richardson	natural	3	2	1	0	1	0	0
Flagstaff	Reservoir-RSR	68	25	12	0	2	2	8
Gull	natural	1	1	1	0	1	0	0
Haley	spring	1	1	0	0	0	0	0
John’s	spring	1	1	0	0	0	0	0
Kennebago	drainage	1	8	1	0	1	0	0
Lincoln	natural	2	2	2	2	0	0	0
Little Beaver	spring	5	1	1	0	1	0	0
Little Jim	natural	2	1	1	1	0	0	0
Little Kennebago	natural	1	1	1	0	1	0	0
Little Lobster	drainage	4	1	1	0	0	0	1
Long	natural	1	1	1	0	0	0	1
Loon	spring	1	1	1	1	0	0	0
Mass Bog	drainage	1	1	1	1	0	0	0
Mooselookmeguntic	Reservoir-RSR	12	20	6	0	6	0	0
Parmachenee	drainage	4	4	1	0	1	0	0
Pepperpot	natural	1	1	0	0	0	0	0
Pond-in-the-River	drainage	1	3	0	0	0	0	0
Quimby	spring	4	1	1	1	0	0	0
Rangeley	Reservoir-RFP	15	7	5	2	3	0	0
Richardson	Reservoir-RSR	15	15	4	0	4	0	0
Round	natural	1	1	0	0	0	0	0
Round Mountain	natural	1	1	0	0	0	0	0
Saddleback	natural	1	2	1	0	1	0	0
Sandy River	natural	1	1	1	1	0	0	0
Shallow	natural	1	1	0	0	0	0	0
Sturtevant	natural	1	1	1	0	0	0	1
Tea	natural	1	1	0	0	0	0	0
Umbagog	Reservoir-RFP	105	31	16	1	13	2	0
West Richardson	natural	2	2	1	0	0	0	1
Wyman	Reservoir-RPR	42	7	7	0	0	5	2
Rangeley Area	**(43 total lakes)**	**515**	**185**	**91**	**15**	**43**	**14**	**19**
New Hampshire*	**(36 total lakes)**	**242**	**38**	**38**	**18**	**3**	**11**	**6**
TOTAL	**79 lakes**	**748**	**223**	**129**	**33**	**46**	**25**	**25**

* “New Hampshire” lakes are those outside of the Rangeley Lakes Study area that were not monitored by other biologists during the 2000 season. New Hampshire lakes include Akers (2 territories), Ayers, Baptist, Big Brook Bog, Big Dummer, Brown Owl, Chocorua, Dan Hole, Deering, Duncan, Grafton, Horn, Ivanhoe, Martin Meadow, Middle Pea Porridge, Massabesic (2 territories), May-Butterfield, Mendums, Millen, Moose, Pemigewassett, Pontook, Red Hill, Reservoir, Round,

Sessions, Spectacle, Sunrise, Swain's, Tower Hill, Upper Kimball, Walker, Waukeena, White Oak, Whitton, Willard, Winnisquam.

Methods

Although we monitored 223 territorial pairs from 1998-2000, the sampling basis for the following six parameters varied geographically and temporally.

1. Collection of tissues for exposure assessment

Methods for handling samples for toxicological analysis: Blood was drawn from the metatarsal vein through a leur adapter directly into 5-10 cc vacutainers with sodium heparin (green tops) and placed immediately on ice in a cooler. Vacutainers were opened once, 10-14 hours later, to add 10% buffered formalin (1:20 formalin-blood ratio) using USFWS protocols (Stafford and Stickel 1981, Wiemeyer et al. 1984). Each time, formalin was drawn from a sealed container with a new one cc syringe with a measurement precision of 0.02 cc. The vacutainer with blood preserved with formalin was then placed in a refrigerator and not opened until reaching the lab. Whole blood from samples less than one cc was immediately frozen and vacutainers not opened until analysis. Feathers were clipped at the calamus and placed in a polyethylene bag. Methylmercury is locked in the keratin proteins in the feather and is not subject to degradation (Thompson 1996). Feathers were clipped again at a standard location at the the superior umbilicus and cleaned in the lab to remove external contaminants.

When possible, BRI biologists collected whole eggs from nests that had been abandoned, predated or flooded. Eggs were only removed from a site when the adults were no longer incubating them, or they were determined inviable (i.e., strong odor, or indications that eggs were not turned). Eggs were placed in a polyethylene bag and labeled with lake and territory name, date, and collector while in the field. Eggs were then frozen as soon as possible. Later, eggs were measured for length, weight, and volume. Egg volume was measured by water displacement and weighed on an electronic balance to the nearest 0.001g. Egg weight was also measured to the nearest 0.001g. The egg length and width were measured with calipers to the nearest 0.001 mm. Eggs were cut open with a scalpel and the contents were placed into sterile I-Chem® jars (including as much of the egg membrane as possible). The contents were then categorized into one of the developmental stages (Table 2).

All samples were labeled in the field within a standard protocol including date, species, age, sex, band or identification number, lake and specific locale, and state. In the field lab, samples were listed on a form and another label was made based on the field form, compared with the field label, and added to the sample (therefore all samples were double labeled). A catalog was developed in the field and a proofed copy accompanied the samples when sent to the analytical lab and again were proofed before preparation for analysis-creating a secure chain of custody of samples.

Analytical methods for blood and feather follow those of Evers et al. (1998a) and meet requirements used by the USFWS and USEPA. All blood and feather samples were analyzed using cold-vapor atomic absorption (CVAA) spectroscopy at the University of Pennsylvania's toxicology lab and for eggs at Texas A&M University Trace Element Research Lab. Analysis of bird blood, feather, and eggs was for total Hg because MeHg comprises 95% or more of the total Hg (Thompson 1996, BRI Unpubl. data). Preparation and analysis of egg contents and fish for Hg concentrations were similar to those used for blood homogenizing and digestion. All eggs were adjusted for moisture loss by dividing the total egg weight by the egg volume (Stickel et al. 1973). Fish Hg body burdens comprise around 85% or more of MeHg.

Analyzer Performance evaluation: Instrument performance was evaluated through use of control charts. Analytical precision of total mercury was calculated as the RSD for three or more replicate analyses. The precision objective was ±10% for total mercury. Accuracy for total mercury was assessed by recovery of matrix spikes and standard reference materials (DORM 2, NRC, Canada). The accuracy objective was 100±10% for spikes that are at least 10x the unspiked sample concentration. Data quality indicators were calculated as described in "Preparing Perfect Project Plans", EPA/600/9-89/087, October 1989. For mercury analyses, 10% of samples were split into laboratory duplicates. Random field blanks were included in each analytical run. During each day's operation, at least 5% of the samples analyzed were distilled water blanks, matrix spikes, or known standards. The analytical detection limit for total mercury in whole blood, feathers and eggs was 25 ppb. Instrument performance was permanently recorded in instrument log books, which include a control chart for tracking instrument performance.

Table 2. Embryological developmental scale used for Common Loon eggs.

NA (not assessable): Developmental stage could not be determined. Contents were gray or yellowish-tan in color and typically had a foul smell. A darker color suggested some degree of development had occurred, whereas a yellow homogeneous liquid may be sifted through and if no dark spots or hardened areas were found we classified the egg as infertile (0).

0: No development was evident. Egg had a yellow/orange or yellow/tan yolk (intact or broken down into a liquid). A translucent jelly-like mass surrounded the yolk sac and showed no sign of embryonic development (e.g. mass not dark or hardened).

1: Embryo was viable (length was up to 1.5 cm). The jelly like mass (embryo) was dense and hardened. Small dark (red) eye spots may be visible at this stage.

2: Developing embryo (length was 1.5 – 2.0) has an apparent central nervous system. Cranial development and visible eyes are apparent. Feathers are absent.

3: The embryo shows advanced development (length was 2-3 cm). Bill was developed (e.g. egg tooth present but soft). Legs and wings were visible but not fully developed. Some feathers were present (first seen in tail).

4: The fully developed embryo was completely covered by feathers. Appendages were completely developed. Vent, preen gland was visible. A small portion of yolk sac remained attached to belly.

2. Assays related to physiological measurements

Five blood parameters were measured in adult and juvenile blood from 1995-98 at sites throughout North America: packed cell volume, glucose, total solids, white blood cell counts, and white blood cell types (differentials). Standard methods were used for each measurement. Corticosterone, testosterone, and estrogen levels were measured with standard assays by Tufts University.

For determining feather asymmetry we cut the second secondary at a standard site on the rachis where the calamus meets the feather's vane to standardize measurements of length and weight. The precision of measurements was 0.001 grams (weight) and 0.01 mm (length).

3. Collection of Behavioral Information

In 1998 (n=753 hours), 1999 (n=571 hours), and 2000 (n=216 hours) we collected a total of 1,540 hours of adult and chick loon behavioral observations from 43 territories on 10 lakes in Maine (and five territories in New Hampshire) between early May and late August for the loon's pre-nesting, nesting, and post-nesting periods. Loon territories were placed into extra high, high, moderate, and low risk categories (Table 1).

We collected behavior data using time-activity budget (TAB) methods based on those described by Altmann (1974), Tacha et al. (1985), and Nocera and Taylor (1998). Observation periods were not staggered throughout the photoperiod because Evers (1994), Mager (1995), Gostomski and Evers (1998), and Paruk (1999) found minimal or no significant relationships between time of day and loon behaviors.

Individual loons were observed in one-hour time blocks for up to five hours/day using a 15-45X spotting scope and 10X binoculars. Observers (who would continually monitor behavior through a spotting scope) relayed behaviors to a recorder, who noted times from a digital stopwatch and recorded categorized observations on data sheets. Observers and recorders also monitored for intruding and non-intruding loons, boats, and predators. In 1998 and 1999, data were collected by six BRI biologists and trained EarthWatch Institute volunteers. Martin and Bateson (1993) addressed potential problems with observer bias and misinterpretation of behaviors. Observer bias was minimized each year by training all BRI biologists simultaneously for 3-4 days, and meeting several times throughout the season. EarthWatch Institute volunteers, although trained, were generally designated as recorders and observed only with the supervision of BRI biologists. Bradley (1985) addressed the importance of minimizing visibility and discovery bias when collecting TABs. We addressed these potential biases by concealing ourselves and/or through remote observation (up to 300 m).

Explanation of Time-Activity Budget Methods

The time-activity budget method is a continuous sampling method (Tacha et al. 1985) which better represents and quantifies behavior data than either instantaneous and/or nonfocal animal sampling methods (Martin and Bateson 1993). Behavior data was classified into two different categories: behavior states and behavior events (Altmann 1974, Tacha et al. 1985, and Nocera and Taylor 1998).

Behavior states were defined as behaviors lasting longer than 20 seconds. Examples of commonly seen adult behavior states were foraging (for self or chicks), locomotion, preening, sleeping, nest sitting, drifting and brooding. Commonly observed chick behavior states included swimming, sleeping, back riding, under wing and preening (Appendix I).

Behavior events were counted behaviors that lasted less than 20 seconds. Events that lasted more than 20 seconds were then timed as behavior states. Examples of common adult behavior events were wing flaps, egg turning, chick feeding, peering, and foot waggles. Commonly seen chick behavior events included foot waggles, head shakes, peers and wing flaps (Appendix I).

There are different ways to categorize and analyze periods when the loon was not visible. Evers (1994) and Gostomski and Evers (1998) collected TABs within one hours continuous time blocks. During a TAB, loons that could not be viewed were classified as “out-of-sight”. They assumed loon behaviors were not significantly different than in-sight behaviors, which avoided potential observer bias. In this study, we collected behavior data continuously until a total of 60 minutes was recorded per individual except in cases where the subject was out of sight for more than 20 minutes. Observation periods that were less than 40 min per individual were not used in the final analysis. Therefore, although the “out-of-sight” behavior category was not incorporated, 60 minutes of continuous observation was generally not made.

In cases where observers felt they could not adequately record behaviors of all target loons present, selected individuals were excluded from observations according to priority. Chick behaviors held the highest priority, while adults were second, based on chick behavioral effects documented by Nocera and Taylor (1998).

Distinguishing/identifying individuals

In order to compare behaviors among individuals in different mercury risk categories (extra-high, high, moderate, and low), absolute identity of the individual being observed was crucial. The potential for confusion of individuals within a territorial pair was much higher than between different pairs due to exclusive territorial behavior and high site fidelity (Evers 2001).

Adults: In almost all cases, at least one or both individuals of the territorial pair were marked (color banded and bill and/or feather marked), which allowed consistent positive identification of the loon being observed. Bill paint and feather markings were often visible from a great distance. Colored leg bands were easily observed during nest sitting and comfort/maintenance behaviors, and were often visible underwater while swimming and foraging with binoculars or a spotting scope. Individuals that were not positively identified were classified as unknown and were not used in the final analysis.

Chicks: Most of the observed territorial pairs hatched only one chick or only one chick survived the first few weeks. These cases tended to have less logistical complications associated with positive and consistent identification of individuals throughout the duration of the TAB than two-chick families. Individuals from the same clutch will typically stay within close proximity to each other and are essentially identical from our observation range. Observers performed TABs on either chick or both, depending on the number of available observers and recorders. We addressed the possibility of confusing the two chicks during the observation period in one of two ways. Whenever possible, one chick was captured and either banded (if > 3 ½ weeks), and/or bill marked (with a orange paint that would degrade in several weeks) so that both individuals could be easily distinguished from a distance. When chicks were not marked, we chose one chick randomly whenever we lost track of an individual, and then continued observations.

Nest Sitting: Monitoring with Data Loggers

Temperature data loggers were used in 1998 and 1999 to discern adult behavioral effects as related to nesting behavior. Deployment sites were chosen according to logistics and designated mercury risks categories—a range of low and high sites were selected. Two types of BoxCar® Pro (Onset Computer Corporation, Bourne, MA) data loggers were used. The Stowaway® XTI logger has a single temperature sensor at the end of a flexible wire, while the Hobo® H8 Pro Series Logger has a dual monitoring system. This system involves one sensor at the base of the unit to monitor ambient temperature, and one sensor at the end of a flexible wire to monitor nest temperature.

The main unit of the data loggers was placed at a site near the nest that received a similar amount of direct sunlight as the nest. Temperature data loggers were inserted into nests that already contained eggs. The flexible probes were inserted into the nest by coming up through the bottom of the nest material and placed 1-2 cm below the egg within the nest. Post-installment nest checks confirmed the temperature sensors were held firmly in place by the covering material. The data loggers were programmed to take temperature measurements every 40 seconds, and were deployed for 1 to 19 days. Information from the data loggers was interpreted using overlapping TABs.

4. Impacts on Individual Survival

We attempted to determine impacts on adult and juvenile individual survival in two ways: 1) regular visits to territories until ice-on and 2) recapturing previously marked adults. Regular visits to territories throughout the season gave limited but useful insights into individual survival. Through consecutive visits to a territory, we were able to evaluate risks associated with that location (e.g. predators, intruders, high wave action) and continually determine the status of the territorial pair and their young.

Recapturing banded and sampled adults from previous years permitted an investigation of Hg accumulation rates. Whereas blood mercury reflects recent dietary uptake, feather mercury levels are more indicative of chronic accumulation rates (Burger 1993).

The chick age class distinction for the behavioral TABs is designed to follow the developing loon's molt pattern, which affects the amount of mercury available in the bloodstream. Young-of-the-year loons feed on lakes for their first 14-20 weeks and 4-10 weeks of that time is after their last feather molt (depending on ice-on). Mercury is depurated into these newly molted gray feathers, thereby lessening the overall body burden of mercury (Burger 1993). Therefore, we captured young loons (1) at different periods of their molt from similar Hg risk areas of Aziscohos and Flagstaff lakes (1994-99) and (2) within the same year to document bioaccumulation rates from their natal lake.

5. Techniques and Definitions for Reproductive Measures

We surveyed nesting and non-nesting territorial loon pairs on 102 territories from ice off (early May) until mid-September (Table 1). Surveys consisted of locating loon pairs every 3-5 days from a boat with 10x binoculars, documenting territorial duration, nest attempts, number of eggs laid, incubation efforts, and causes for nest abandonment/failure. Human disturbance, evidence of predators, and frequency of intruding loons were also documented at this time. Reproductive information from New Hampshire was based on a 25-year productivity database from the Loon Preservation Committee (Taylor pers. com.).

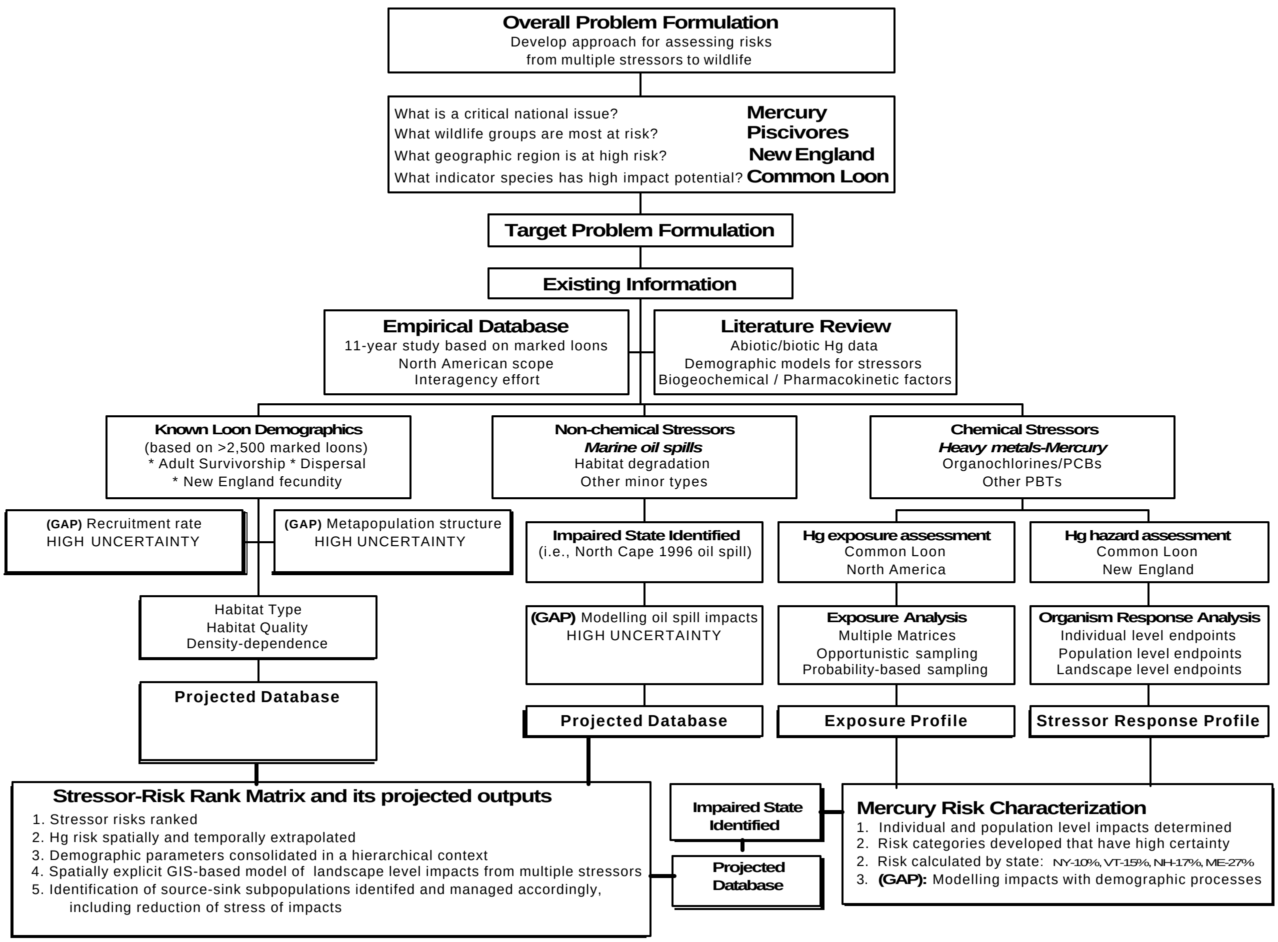
We collected four reproductive parameters from each territory and they include: (1) presence of territorial pair, (2) nesting attempts, (3) hatching success and (4) fledging success. Determining whether a loon laid an egg was the most time intensive parameter. Because territories could not always be monitored within our standardized guideline, we could not always confirm whether an egg was laid and therefore we did not use those territories for the nesting pair parameter during our overall productivity analysis. Successful fledging was defined as young loons reaching the age 6 weeks or older. This is consistent with most national loon population monitoring programs.

Results and Discussions

The following description and interpretation of MeHg impacts on the Common Loon is the third year of a multi-year effort. The construction of this large temporal and spatial database will facilitate and improve national wildlife risk assessments by federal and state government agencies. Perspective for the MeHg exposure levels to loons including one of their favored prey, yellow perch, is initially provided and then followed by the assessment of MeHg hazards to loons through the investigation of their relationship with physiological, behavioral, survival, and reproductive measurements. The beginnings of a risk characterization that is built from the exposure and hazard assessments provide insight into the population level impacts of MeHg to piscivorous wildlife such as the Common Loon.

The overarching goal of this project is designed to (1) assemble the wealth of existing toxicological and demographic information into an integrated format, (2) fill in data gaps identified through demographic models and evaluation of current inadequate sample sizes, (3) improve resolution of spatially explicit toxicological and demographic information through analysis of genetic population structure of the Northeast metapopulation, and (4) organize the existing and newly collected databases into a stressor-risk rank matrix that will provide a basis for spatially-explicit models of landscape level impacts from critical environmental stressors such as MeHg availability and marine oil spills (Figure 2).

FIGURE 2. Conceptual framework for bioassessment model of multiple stressors of the Common Loon.



A. Exposure Assessment

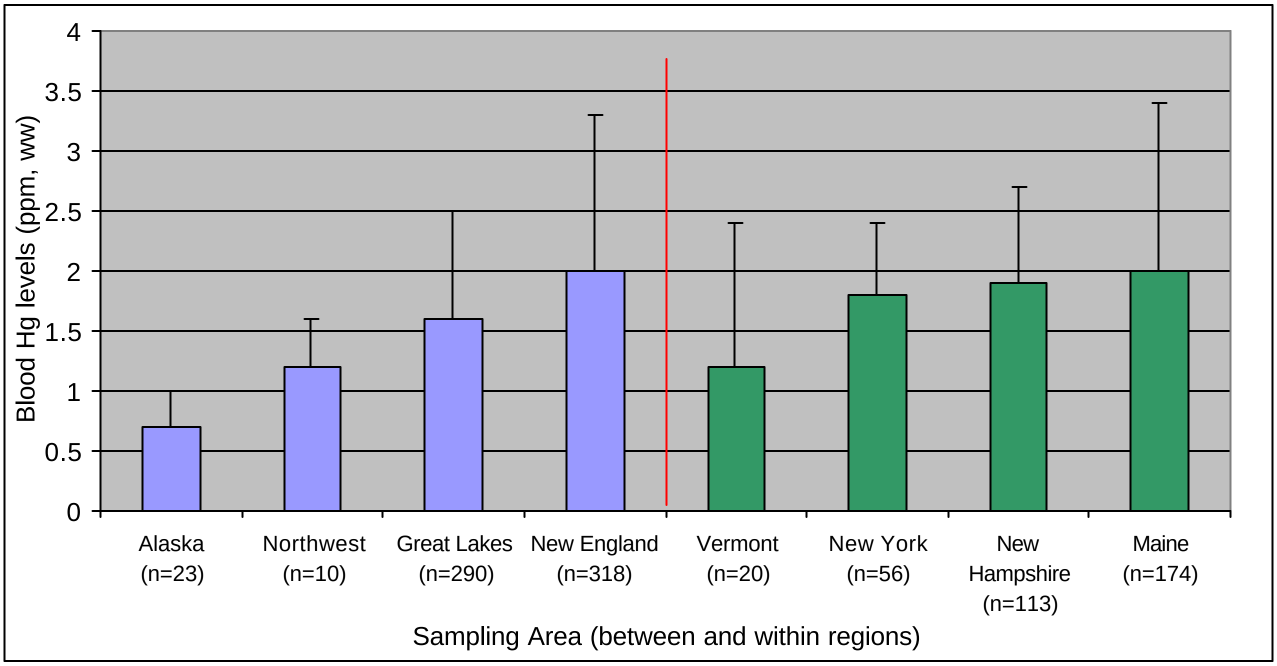
1. Common Loon Mercury Profile

In recognition of widespread environmental contaminants, the United States Environmental Protection Agency (EPA) uses the Environmental Monitoring and Assessment Program (EMAP) as a long-term tool for monitoring and assessing ecological condition (e.g., effectiveness of the Clean Air Act). The monitoring of surface waters using EMAP's probability-based surveys for ecological indicators provides a statistically valid technique for making regional and eventually national extrapolations of the exposure and effects of various environmental stressors (e.g., Whittier et al. 1997, Yeardley et al. 1998). The complementary regional program, REMAP, has also proven effective in this same regard (e.g., Stafford and Haines 1997, Mower et al. 1997). EMAP and REMAP efforts within Region 1 and elsewhere are designed to provide a method for evaluating and prioritizing the threat of environmental stressors to lacustrine habitats. One issue that has surfaced from these studies is the widespread and elevated levels of Hg in sediments, water column, fish tissue, and piscivorous wildlife.

Although the sampling of loons and other biota from 1992-00 indicates geographic differences in MeHg availability has a west to east trend across North America, with New England having the highest levels (Figure 3)(Evers et al. 1998a), within-region differences are primarily related to hydrological and biogeochemical factors (Evers and Reaman 1998). Within-region loon blood Hg levels appear to be similar in Maine, New Hampshire, and New York and tend to be lower in Vermont. Particularly high levels of MeHg bioavailability are in the western Adirondack Mountains of New York, in southeastern New Hampshire, and in several areas of Maine (Figure 4). Because of these factors and potential point sources, a geographic risk assessment using EMAP/REMAP protocols needs to incorporate and characterize sampling areas in a probability-based strategy.

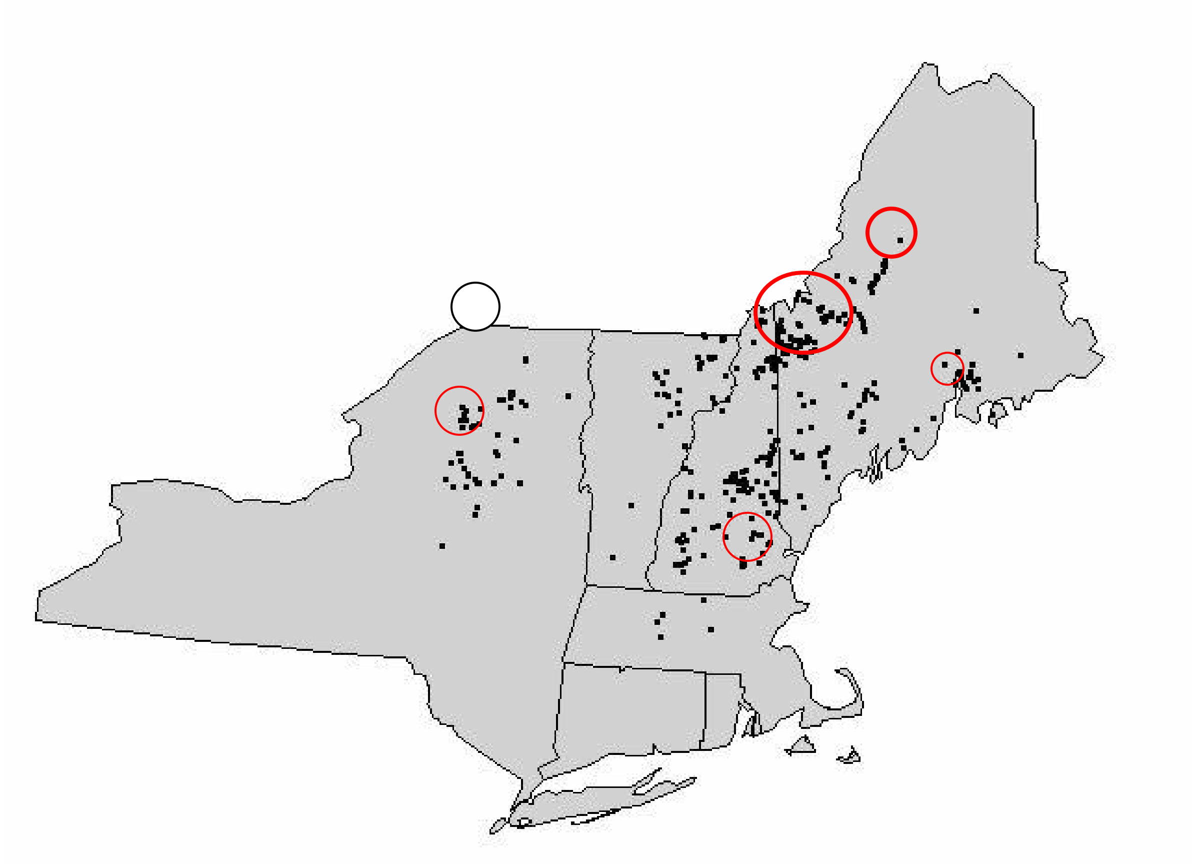
Until EMAP/REMAP protocols can be applied across the New England landscape, we have used an iterative, opportunistic sampling approach for the past eight years. During this time, we collected 431 samples of three loon matrices across much of Maine south of Bangor (Figure 4). These Hg levels have been linked with risk categories developed by this study (see Risk Characterization). A cumulative assessment of Maine’s breeding loon population shows that 28% of the breeding population is at high risk to Hg poisoning by impacting overall reproductive success, behavior, and survival (see risk characterization section).

Figure 3. Mercury levels measured in loon blood in U.S. regions and within New England.



Blood Hg levels (ppm, ww)
0
0.5
1
1.5
2
2.5
3
3.5
4
Alaska (n=23)
Northwest (n=10)
Great Lakes (n=290)
New England (n=318)
Vermont (n=20)
New York (n=56)
New Hampshire (n=113)
Maine (n=174)
Sampling Area (between and within regions)

Figure 4. Distribution of MeHg bioavailability in New England based on loon blood Hg levels with hotspots



identified by red circles.

2. Yellow Perch Mercury Profile

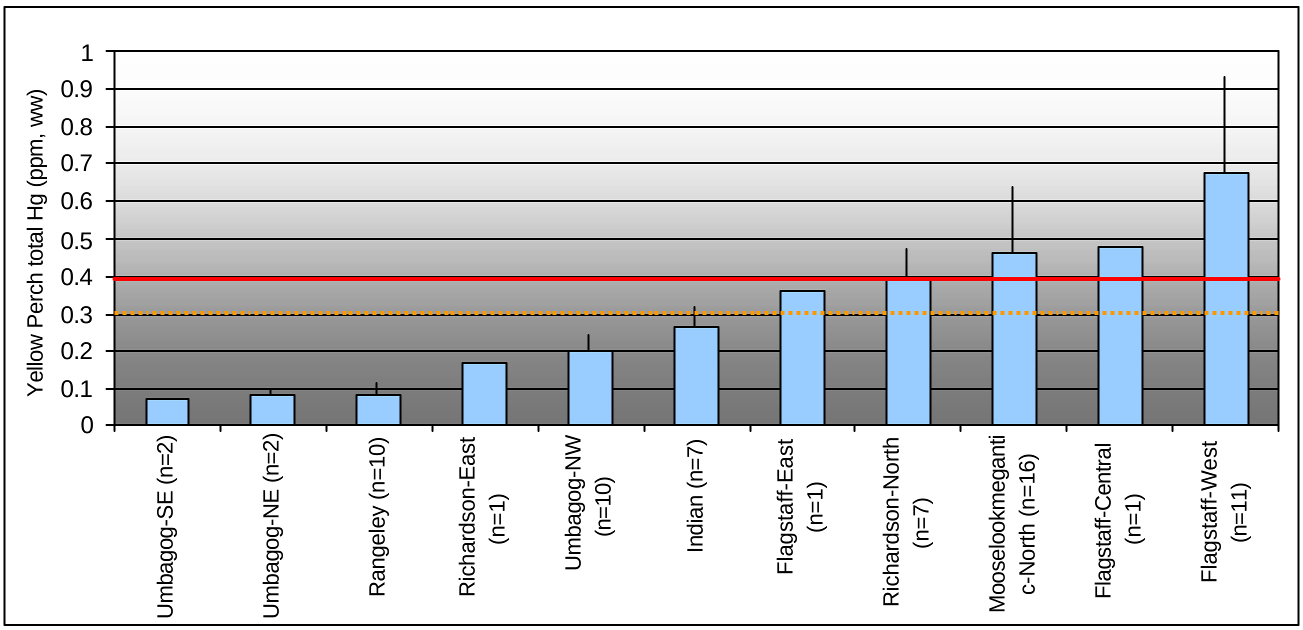
We measured Hg in loon prey items to determine 1) Hg concentrations in potential loon prey and 2) their correlation with adult blood Hg concentrations to confirm the Hg biomagnification pathway and 3) whether certain prey items have higher Hg concentrations and pose a greater risk to loons.

To determine prey Hg concentrations in species and size categories that loons select, we developed a collection protocol based on a study by Barr (1996). We found adult and juvenile loons selected small- to medium-sized fish. Large fish (over 15 cm) were preferred but their capture availability was lower than other size classes. In Ontario, adult loons consumed 432g (small fish), 365g (medium-sized fish), and 163g (large fish) daily or approximately 20% of their body weight per day. The percentage of individual fish consumed by size class was: 80% small, 18% medium, and 2% large. Male loons from Maine and New Hampshire are 26% larger than their counterparts in western Ontario (geographic differences in female body mass are less). We adjusted the amount of fish consumed by the heavier New England loons to be: 45%,38%,17% for females and 33%,40%,27%, for males.

Barr (1996) determined that because of their "zig zag" swimming behavior, Yellow Perch (*Perca flavescens*) were the preferred food of adult and juvenile loons. Median size perch consumed during Barr's trials for 12-15 week old loons (adult sized individuals) was 5.0cm and ranged from 1.0-9.5cm. Adult loons commonly ate perch up to 12.0-16.0cm.

Barr (1986) found the lowest observed adverse effect level (LOAEL) for adult loons feeding on fish with whole body Hg concentrations over 0.30 ppm (i.e., impaired reproduction) and 0.40 ppm (i.e., no reproduction). Barr did not specify daily dietary Hg ingestion thresholds in his study, but it is assumed that loons in this contaminated system were regularly foraging on fish at and above this level on a regular basis. Loon prey Hg data from 1996-97 indicate that mean Yellow Perch Hg concentrations sampled in many natural lakes and reservoirs from Maine were comparable with Barr's contaminated site and above the level he found to cause reproductive impairment (Figure 5).

Figure 5. Yellow perch Hg exposure levels in targeted lakes of the Rangeley Lakes study area. Because



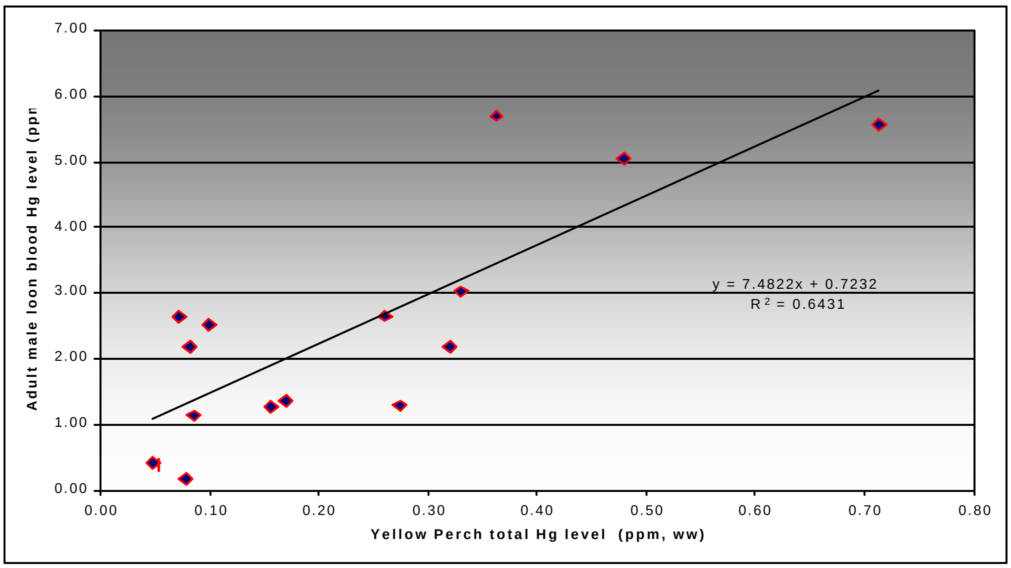
MeHg bioavailability usually differs across a water body, large lakes were separated into quadrants.

Yellow perch have been shown to be the preferred prey item in the Rangeley Lakes area. Evers and Reaman (1998) and additional datasets (Figure 6) show strong relationships between loon blood and 10-15 cm yellow perch total Hg levels. Burgess et al. (1998) also found a strong relationship between loon blood and similar-size class yellow perch Hg levels (r^2=0.79). An understanding of the loon-prey Hg relationship provides insight into the pathways and pharmacokinetics of MeHg. This understanding and sampling efforts of loons on their wintering waters has shown that the majority of Hg in the loon's blood is from its summer diet.

There are important qualifiers to the loon-perch Hg relationship that require further exploration before we are able to confidently predict the impacts of MeHg bioavailability to upper trophic level wildlife. Although loons prefer perch, increased fish composition can severely confound these predictions because loons are more likely to opportunistically forage on other prey items (e.g., sucker Hg levels can be an order of magnitude lower than found in same-size perch in the same area of water). Secondly, behavioral changes of loons and their prey that are related to exceptionally high MeHg levels may alter predictable predator-prey relationships. Studies have shown elevated Hg levels may cause fish to change their behaviors in ways that may make them more or less available to predation (Kania and O'Hara 1974, Weis and Weis 1995, Ososkov and Weis 1996, Schwartz 1998). Significant aberrant deviations from normal swimming (e.g., slower sustained swimming times) and escape behaviors (e.g., enhanced zig-zag patterns) were measured in 10-15 cm yellow perch ranging from .08 to 0.39 ppm of Hg. Kania and O'Hara (1974) found significant behavioral alterations in fish with 0.67 ppm.

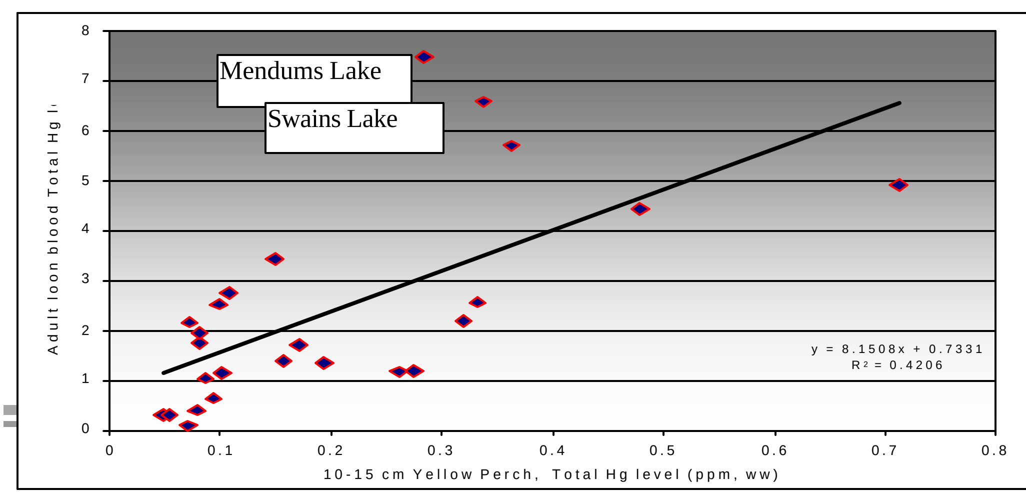
The biotic Hg levels of two New Hampshire lakes (Mendums and Swains) that are downwind of highly Hg laden emission plumes are exceptionally high, however, loon blood Hg levels are far more elevated than perch Hg levels indicate (Figure 7). The lack of agreement is difficult to explain, but the loon diet does not appear to be based on perch and if only perch were used to indicate aquatic Hg loads, those lakes would not be considered as high of a risk to wildlife.

Figure 6. Relationship between adult loon blood and yellow perch Hg levels (total Hg, ppm, ww) for 16 lakes



in New England.

Figure 7. Relationship between adult loon blood and yellow perch Hg levels (total Hg, ppm, ww) for 18 lakes in New England, including two highly contaminated lakes in New Hampshire, Swains and Mendums.



B. Hazard Assessment

We believe investigating multiple levels of biological organization (e.g., genetic, individual, and population) for the Common Loon provides the quantitative benchmarks needed to evaluate environmental stressors like Hg. Once this hazard can be quantified the extent of exposure can be assessed through risk characterization techniques. The following sections describe our techniques for assessing hazard for six targeted parameters and partly follow recommendations by Peakall (1992).

1. Physiological Relationship with Mercury

The measurement of various blood chemistry parameters and hormones provide a way for assessing an organism's health. They also can be used as biomarkers that can demonstrate the presence and extent of contaminant exposure to an organism and predict potential impacts on that individual (Bensen et al. 1990). For example, Frederick et al. (1997) found a relationship between decreased packed cell volume (PCV) and elevated Hg levels in dosed egrets. Colburn et al. (1993) identified Hg as an endocrine-disrupter and since the loon's body burden of Hg nears the highest tested levels for wildlife in freshwater systems we measured testosterone and estrogen levels. Corticosterone hormones are released during periods of stress and are being increasingly used as indicators of environmental stressors (Astheimer et al. 1992, Smith et al. 1994), including Hg (Friedmann et al. 1996).

a. Blood Profiles

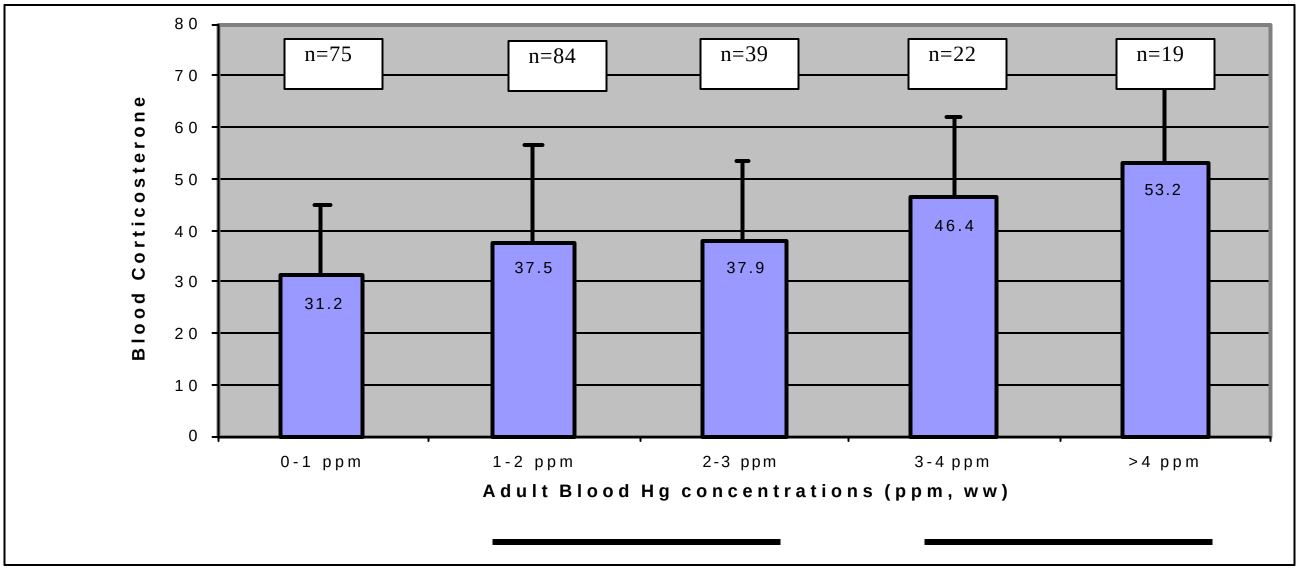
From 1994-99, Tufts University and BioDiversity Research Institute have collaborated to develop suitable and logistically simple biomarkers for Hg and to determine reference levels of various hematological parameters in the loon. Over 200 adults and 100 juvenile loons have been sampled. Blood profiles have commonly been used in birds as measures of the impact of Hg on an individual's overall health (Frederick et al. 1997, Hoffman and Heinz 1998, Wolfe and Norman 1998). Statistically significant relationships with several blood parameters have been detected with increasing MeHg levels (Frederick et al. 1997, Hoffman and Heinz 1998).

We measured packed cell volume, number and type of white blood cells, and plasma protein. We did not find a significant relationship between elevated Hg concentrations and PCVs ($p>0.5$), white blood cell abundance ($p>0.5$), or white blood cell types ($p>0.05$). Other studies found relationships in birds (all dosed in captive situations) with Hg levels higher than those found in our wild loon populations (Frederick et al. 1997). We did detect a nonsignificant, but lower variability in white blood cell counts in loons with higher Hg levels.

b. Hormones

Although several studies have demonstrated biotic relationships with Hg levels and cortisol stress hormones (Friedmann et al. 1996, Hontela et al. 1992, 1995) those relationships have not been found in birds. BRI and their collaborators at Tufts University School of Veterinary Medicine have recently developed radioimmunoassays for measuring circulating corticosterone levels in loons. Corticosterone is released in response to stressful stimuli and can potentially provide evidence of immunosuppression. There are many confounding factors when comparing corticosterone and MeHg levels, such as handling stress, reproductive stress, and nutritional stress (Sturkie 1986).

Figure 8. Mean concentration of corticosterone versus blood Hg concentrations in adult Common Loons from selected sites in North America (bars represent mean +/- 1 SD)*.



* Those blood Hg concentration categories not connected with a line are significantly different from one another.

During 1995-1999, we collected plasma from 239 adult loons and analyzed the levels of corticosterone. Sturkie (1986) reported ranges of 0.4 to 29 ng/ml for non-stressed birds. We found corticosterone levels to be elevated (i.e., >30 ng/ml) in 88% of the individuals, which ranged from 8.2 to 100.2 ng/ml. Evers (2001) found loon circulating corticosterone levels to be independent of the amount of handling time before taking a blood sample. Therefore, although our elevated levels are partly attributed to the stress of capture, the circulating corticosterone levels we are measuring are independent of capture and handling times. Loons in the low Hg risk category (0-1 ppm in the blood) represent our reference condition.

When comparing the mean of circulating corticosterone levels with separate categories of increasing one ppm of blood Hg levels, there is a significant increase (at least a $p<0.05$) between low (0-1 ppm) and moderate (1-3 ppm) Hg levels, moderate and high (3-4 ppm), and high and extra high (>4ppm) (Figure 8, Table 3). Therefore, although capture of adult loons with nightlighting methods and the ensuing 30-45 minute handling time does initiate a physiological reaction, stress in our loons with the highest body burdens of Hg does not solely reflect our capture impacts. It appears that blood Hg concentrations have a significant positive correlation with elevated circulating corticosterone levels and that these relationships agree with our established Hg risk categories.

Table 3. Statistical probability matrix for 5 categories of blood Hg concentrations and their relationship with corticosterone levels in adult Common Loons.

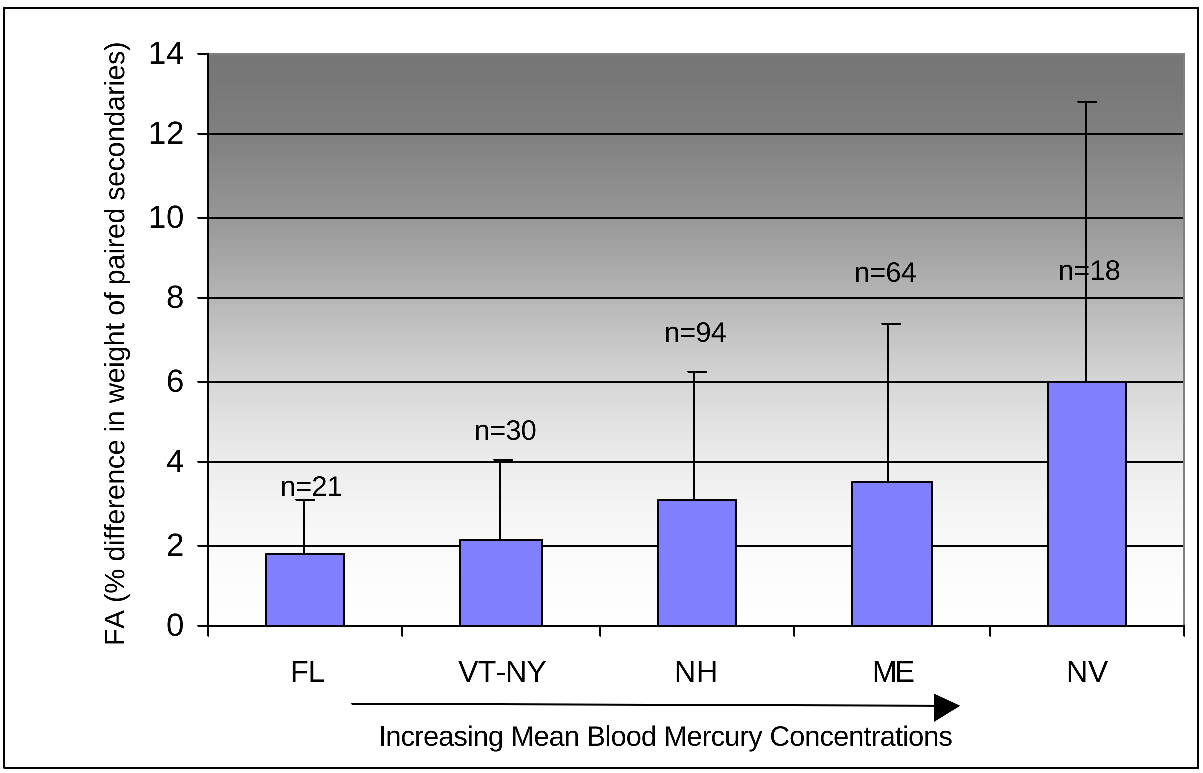
Category	Low	Mod-1	Mod-2	High	XHigh
0-1 ppm	-	p=0.03	p=0.03	p<0.01	p<0.001
1-2 ppm	p=0.03	-	NS	p=0.05	p=0.002
2-3 ppm	P=0.03	NS	-	p=0.05	p=0.002
3-4 ppm	p<0.01	p=0.05	p=0.05	-	NS
> 4 ppm	p<0.001	p=0.002	p=0.002	NS	-

c. Developmental Stability

We measured the relationship between lifetime Hg body burden and fluctuating asymmetry (FA). Clarke (1995) considered the ability of an individual to develop bilateral characters to be one of the best estimates of developmental stability—an indirect measure of fitness. Because feather growth is linked with the very protein reserves that are associated with bound-MeHg in the muscle tissue (Murphy 1996, Scheuhammer 1991), it is likely that remobilization of MeHg coincides with the proteins used for feather formation. Clarke (1995) and Polak and Trivers (1994) suggested fluctuating asymmetry to be a sensitive measure of long-term body condition and Yablokov (1986) and Moller and Swaddle (1997) both considered FA as a sentinel for subtle environmental perturbations prior to visible effects in population viability.

Analysis of 227 paired feathers collected and measured in 1998-99 indicates breeding populations with higher mean feather Hg concentrations to be significantly more asymmetrical than those populations with lower levels (Figure 9). We compared differences in the weight and length of paired second secondaries and feather Hg levels. Because weights had less of a measurement error than length, although they were strongly correlated (r^2=0.86), we used differences of paired feather

Figure 9. Geographic differences in developmental stability measured through fluctuating asymmetry of Common Loon second secondaries.



weights as measures of fluctuating asymmetry. Differences in paired feather weights were not significantly correlated with feather Hg levels ($p>0.05$), however, when we pooled individuals according to state as an indicator of Hg stress to breeding populations, New England (Maine and New Hampshire) breeding adults had significantly more flight feather asymmetry than breeding populations with significantly lower feather Hg levels ($p<0.05$). Feather samples from Florida likely represent breeding adults from the upper Great Lakes (Evers et al. 2000), which also have mean blood Hg levels significantly lower than New England loons (Evers et al. 1998a). Migrant loons staging on Nevada's Walker Lake further provide confidence in this analysis. The 26 loon blood samples had a mean Hg level of 3.24 ppm, which is significantly higher than Maine's mean adult blood Hg level of 2.38 ppm. FA in the Nevada loon's feathers was significantly higher than what we found in New England ($p<0.05$).

It appears that the loon's remiges are a sensitive indicator of FA and the relationship of FA with high Hg risk breeding loon populations potentially makes this bioassay technique important for monitoring aquatic integrity. Although other stressors may disrupt developmental homeostasis, and genetic diversity (especially in the loon, e.g., Dhar et al. 1997) may predispose some populations to have greater FA than others, this technique is as an excellent "catch-all" benchmark for predicting subtle environmental stressors.

2. Behavioral Relationships with Mercury

a. Behavioral relationships with Hg risk

Relating behavior with environmental toxins is a useful indicator of sublethal effects (e.g., Doving 1991). Detection of behavioral abnormalities in relation to an environmental stressor such as mercury gives us insight into the most vulnerable behavioral and physiological mechanisms of nesting and chick-rearing Common Loons that could ultimately impact reproductive success, chick survival and adult survival. Although Nocera and Taylor (1998) and Counard (2001) found subtle but significant behavioral differences related to Hg in loon chicks, similar relationships have not been previously quantified in adults.

Territories within our study were divided into four mercury exposure categories: extra high, high, medium and low based on literature and in situ studies by the authors and their collaborators for five matrices (Table 3). We compared the mean percent of time spent in each behavior among these categories to determine behavioral differences that might be associated with mercury. In 2000, we had the opportunity to study two additional pairs of nesting loons that had Hg levels considerably greater than any of the other individuals in our study (Swain's Lake and Mendums Lake) in the seacoast region of NH. Although the sample size is low, we have included them in our analyses in some cases as a separate "extra-extra high" (XXHi) category because we believe it can be used to effectively gain insight into acute effects of mercury toxicity. In some cases, high and extra high categories were combined due to a low sample size in the high category. Behavioral-Hg relationships are examined by using the data categorically and continuously. Regression analyses employ the mean adult blood mercury values for all territory-holding individuals captured within each territory (regressions specific to gender used a mean of adult blood mercury specific to that gender). In these analyses, each data point represents a loon territory.

b. Geographic and gender differences in behavior

Adult male loons have significantly higher levels of mercury than females (Evers et. al 1998), based on blood Hg concentrations (Figure 25). Because males have significantly higher Hg risk than females and 36% of males in Maine are at risk from mercury exposure (compared to 19% of females), we expected potential differences in gender roles to be biased toward males. Loon behavior data collected in the Midwest showed few significant differences in parental roles and parental effort during pre-incubation, incubation, and post-hatching periods (Evers 1994 [250 hrs], Mager 1995 [1,400 hrs], and Paruk 1999 [4,200 hrs]). We have used these studies as the baseline comparison for the nesting and gender components of our study. Behavioral differences within territorial pairs on our Maine study sites had a greater tendency to differ than those studied in the Midwest.

Table 4. Percent of time spent in eight behavioral categories during three breeding periods in Maine, 1998-1999

	Pre-nest (n=57hrs)		Nesting (n=276hrs)		Post-nest (n=462hrs)	
Activity	Male	Female	Male	Female	Male	Female
Foraging	50	65	19	23	7	7
Resting	10	11	7	4	3	1
Locomotion	13	11	19	17	4	5
Preening	18	4	12	8	2	2
Courtship	1	1	-	-	-	-
Nest Sitting	-	-	39	48	-	-
Chick rearing	-	-	-	-	82	83
Agonistic	9	9	1	1	1	1

Pre-nesting: Evers (1994) found slight gender differences in the percentage of time spent during the pre-nesting period. Males and females did not differ more than four percent in the time spent in the same behavior categories. Our study found more dramatic differences in the allocation of time between the sexes. Males spent a significantly greater percentage of time preening (18% vs. 4%) and less time foraging (50% vs. 60%) than did pre-nesting females. Males also spent slightly more time in locomotion, and slightly less time resting. No differences were apparent between the sexes in the amount of time spent in agonistic behavior or courtship.

Nesting Period: Within the nesting period, we found that adult male loons spent slightly less time foraging (19% vs. 23%) and significantly less time nest sitting (39% vs. 48%) than females (Table 4). Evers (1994) found only slight differences (34% vs. 36% in foraging; and 48% vs. 49% in nest sitting) by gender within the same behavior categories. We found males that were not nest sitting spent more time in preening, locomotion, and resting behaviors than did females. Interestingly, females still spent more time foraging than did males (23%, vs. 19%), regardless of the fact that they spent more time nest sitting. Paruk (1999) noted that nest-sitting loons tended to leave the nest, pant, and flutter the gular and assume the “sprawl” posture (McIntyre 1988) more during unusually hot weather. When these birds left the nest, dives were shallow and closer to the nest. We speculate that nest sitting may have higher energy demands than many behaviors that occur in the water, due to increased thermoregulatory stress. We have found a tendency for males to choose behaviors that have lower energy demands such as locomotion, preening and resting over nest sitting and foraging behaviors, which likely require higher energy demands.

Post Nesting: During the post-nesting period, differences in gender were less apparent than the pre-nesting and nesting periods. Males and females spent the same percentages of their time in foraging, preening, and agonistic behaviors. Slight differences were found in percent time spent in chick rearing, resting, and locomotion between the sexes. Again, males spent more time resting, but slightly less time chick rearing and in locomotion than females.

c. Nesting Period: Behavioral relationships with Hg risk

Time spent on the nest is a key behavior for detecting abnormalities related to MeHg body burdens

(Figure 10). We have documented several cases where high-Hg males have not properly incubated the eggs. Hg has been found in several other studies to have a negative impact on egg laying in loons (Barr 1986, Burgess et al. 1998) and other species (Heinz 1979). In 1999, we reported on aberrant behavior in a high Hg territory. Because incubation has been shown to be equally shared in loons (Evers 1994, Paruk 1999 Mager), and since male Common Loons are known to have higher levels of Hg than females (Evers et al. 1998a) this discrepancy is notable.

Precedence for measurable Common Loon adult behavior abnormalities has been documented during incubation in past years, and evidence indicates reproductive impairment is associated with high mercury exposure (Burgess et al. 1998, Evers et al. 2000, Barr 1986), suggesting elevated Hg concentrations can have an effect on adult behavior and ultimately reproductive success. In this study, we quantified adult behavior through time activity budgets (TABs) during three distinct breeding periods: pre-nesting, nesting and post-nesting. These data were compared to time-activity budgets gathered in the Midwest in Michigan's Seney National Wildlife Refuge (Evers 1994), Isle Royale National Park (Gostomski and Evers 1998), western Upper Peninsula (Mager 1995), and in Wisconsin's Turtle Flambeau-Flowage (Paruk 1999) for differences in behavior. However, biogeographic and geochemical differences between the sites confound these comparisons. We also compared gender differences during the breeding periods and low-Hg sites (controls) with the high-Hg sites in Maine (and some territories from New Hampshire).

Specific cases of aberrant nesting behavior in high mercury individuals

Some behavior abnormalities are difficult to quantify or are not necessarily adequately represented using time-activity budgets. The following two cases of atypical behavior were recorded for individuals identified as having "extra-high" exposure to Hg. These qualitative observations likely represent cases of acute behavioral abnormalities in relation to mercury.

1999: ***Stratton territory, Flagstaff Lake, Maine****. The banded male had a Hg level of 4.00 ppm and the banded female had a Hg level of 3.9 ppm. Two eggs were laid on 1 June and both pair members began incubating. However, after three days, the male no longer incubated the eggs. Mate switches in loons typically occur every 4-6 hours to permit the incubating bird time to forage. On day 4, the female would leave the nest with the male nearby and she would initiate foraging. Instead of the typical behavior of the male moving on the nest to incubate the eggs, he would only guard the nest. The eggs would remain unprotected until the female returned 4-6 hours later. This pattern continued for five days and the nest was then abandoned. Inspection of the abandoned eggs revealed they were fertile but were not viable. This was likely because of a substantial amount of time spent un-incubated. We believe this nest failed due to behavioral abnormalities related to the male's high Hg.*

2000: ***Mendums Lake, NH****: The male and female had blood Hg levels of 8.10 and 6.89, respectively. Observations indicated that one individual disproportionately incubated the eggs. This was never confirmed since the adults were not banded at the time of observations. When the nesting pair was approximately 28 days into the incubation period, the incubating adult was observed to depart the nest (containing two eggs) and the general vicinity. The eggs remained unincubated for a minimum of one hour. No intruding loons or humans appeared to instigate or explain this action. In most cases, nesting loons' fidelity to incubation increases as the incubation period progresses. Although one egg hatched on schedule, the other did not. The adults brooded the first chick and immediately* *abandoned the second egg. The chick disappeared within the first few days of the chick-rearing period.*

Figure 10. Average percent of time spent by adult male and female Common Loons during the nesting period in four Hg exposure categories.

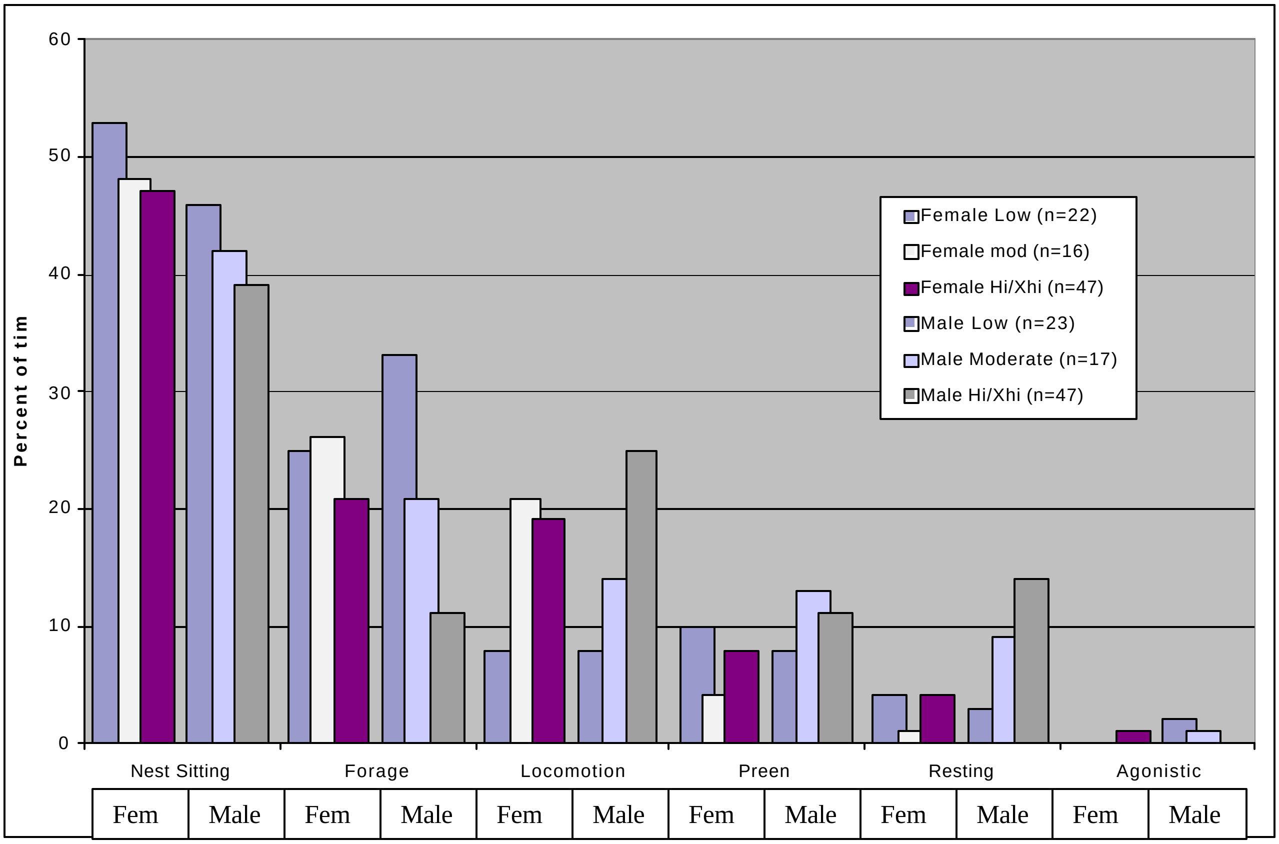


Figure 10 compares behavioral differences between sexes both within and between risk categories. Because males have significantly higher levels of Hg than females, we might expect to find behavioral differences between the sexes. We have compared the percent time spent by nesting loons in six behavior categories by Hg risk category and gender (Figure 10). We have combined the high and extra high risk categories for this analysis due to insufficient sample size in the high-risk category. This combined category will be referred to as "high" herein. We believe the combination of these two categories will result in more conservative or subtle differences when comparing low and moderate risk categories. The following describes significant differences in the percent time nesting loons spent in various behaviors by gender and risk category, representing an analysis of behavioral and gender differences across three Hg exposure categories.

Nesting Period: Nest Sitting

Nest sitting females and males display similar patterns between risk categories. High Hg loons spent less time nest sitting than did moderate-risk individuals, which spent less time nest sitting than low risk loons. When comparing the genders in the behavior of nest sitting, males spent less time than females in every risk category. Evers (1994) and Mager (1995) also reported minor differences in incubation roles between genders, with females tending to nest sit more than males ($p > 0.05$). In addition, it is possible that geographic differences confound comparisons between Maine studies and those of the Midwest. Nonetheless, differences also are apparent between risk categories, suggesting that Hg may be impacting nest sitting behavior. Low mercury risk males and females spent 99% of the time incubating eggs; leaving the eggs unincubated for only 1% of the time sampled. Loons in the moderate risk category incubated for a total of 90%, leaving the eggs unincubated for 10% of the time sampled. High risk males and females spent 86% of their time nest sitting, leaving the eggs unincubated for 14% of the time sampled. Unattended eggs have a higher probability of being predated by avian or mammalian predators, which likely results in a higher incidence of nest failures.

Increased time spent in agonistic behavior by a risk category would represent a confounder in the incubating loon's tendency to nest sit (we have commonly observed loons to prioritize defending their territory over incubating eggs). Time spent in agonistic behavior, however, is relatively low (0-3%) in all risk categories during the nesting period. This level is similar to Evers (1994), who reported less than 1% of time either male or female spent in agonistic behavior. The patterns and proportions of time spent presented in Figure 10 lead us to believe that this time was likely spent in either locomotion and/or resting behaviors, as opposed to foraging for self, preening or agonistic behavior. Evers (1994) reported 5% and 4% (M and F) for both locomotion and resting, compared to our and 25% and 19% (high risk M's and F's) for locomotion, and 4% and 14% (high risk M's and F's) for resting. On the other hand, the amount of time high risk individuals spent preening, foraging for self, and locomotion are similar or lower than reported by Evers (1994) and Gostomski and Evers (1998). We feel that this tendency for a "redistribution" of time from nest sitting to specific behaviors such as locomotion and resting is an important finding.

Nesting Period: Foraging

When analyzing the percent time males spent in other behavior categories, the stepwise pattern observed between risk categories in nest sitting is consistent with that of several others as well, including foraging. Males follow a more dramatic downward step pattern for foraging than females: high Hg loons spent less time foraging than did moderates, which spent less time foraging than the low risk loons. Evers (1994) reported males and females to forage 34% and 36%, respectively, which is similar to low risk males in Maine (33%). High and moderate risk males in our study, however, spent 11% and 21% of their time, respectively, in foraging behavior. Gostomski and Evers (1998) reported on time-activity budgets of loons nesting on Lake Superior, likely representing the most ideal conditions for turbidity and prey abundance. They reported females to forage11% during the nesting period. Although we have not controlled for the confounder of turbidity in our study, we feel that time spent foraging should lie between the levels given by Evers (1994) and Gostomski and Evers (1998). Other studies, such as Bouton et al. (1999), found a significant relationship between mercury and the mean time necessary to capture fish. Dosed Great Egrets (Ardea albus) (0.5mg methyl HgCl/kg) were found to be consistently slower at capturing fish than controls ($p = 0.041$). Significant effects of age and sex were also reported ($p = 0.004$, and $p = 0.004$, respectively), and dosed birds were significantly less likely to eat fish presented to them (even in cases where they caught them) in both camouflage ($p = 0.0003$) and contrasting ($p = 0.003$) pool background settings, potentially suggesting an impact on appetite and/or metabolism. The effects and differences reported with loons in this study agree with preliminary findings by Frederick et al. (1997), who suggests a debilitating effect of Hg on vision in birds. We feel that a large amount of evidence points toward a negative relationship between mercury and foraging behavior and efficiency.

Nesting Period: Preening, Resting, and Locomotion

High mercury loons spent 11% and 8% of their time preening. This is similar to levels reported by Evers (1994) and Gostomski and Evers (1998). Although Counard (2001) and Nocera and Taylor (1998) report significant positive relationships between blood-Hg levels and preening in juvenile loons, we did not find similar relationships in our study for adults or juveniles. Bouton et al. (1999) reported a tendency for dosed Great Egrets to preen more frequently than controls.

Loons in our study spent between 1% and 14% of the time in resting behavior, with moderate and high mercury males displaying the highest proportion of time spent (9% and 14%, respectively). By comparison, Evers (1994) reported 5% (M's) and 4% (F's), and Gostomski and Evers (1998) reported roughly 10%. Because several studies have made reference to relationships between Hg and activity levels (Bouton et al. 1999, Frederick et al. 1997), we feel that increases in this behavior state are of concern and warrant further investigation.

An increasing pattern also occurred for loons in the locomotion behavior state (i.e., swimming). High mercury loons spent more time in locomotion than did moderates, which spent more time in these behaviors than did the low risk loons. High risk males and females in our study tended to spend more time in locomotion (25% for males and 19% for females) than in other studies such as Evers (1994), who reported 5% (M's) and 4% (F's), and Gostomski and Evers (1998), who reported approximately 10%. Counard (2001) found a positive relationship between swimming and blood Hg in juvenile loons greater than 40 days of age.

Our findings emphasize the importance of knowing both the Hg levels and the gender of individuals being studied. Differences were most pronounced between risk categories when analyzed by gender. Many relationships are generally strongest for males as would be expected (Figure 10). In many behaviors such as locomotion and foraging, the female's distribution of time spent approaches that of the male, but there is less consistency in the relationships between risk categories. Our data shows a tendency for increased effect of Hg on males. We believe that this information indicates that the threshold for effects related to Hg lie within the range of our risk categories.

"Extra-extra high" category analysis (Nesting Period)

The "extra-extra-high" (XXHi) category data based on (Mendums and Swains Lakes) could not be separated into sexes due to the number of tab-hours during which the individuals could not be sexed in the field. Mean male and female nest sitting was 46% for XXHi loons, leaving the eggs unincubated for 8% of the time observed. XXHi pairs spent greater proportion of time in higher energy behaviors such as foraging than was expected, and spent no time in drifting or sleeping behaviors (resting category). The Mendums pair was observed on several occasions to display abnormal incubation patterns, leaving the nest unattended for at least an hour days before hatching. Although continual field observations led to suspicions of disproportionate time spent in incubation by one individual, these suspicions could not be confirmed because the pair was not banded during this period (see "Specific cases of aberrant nesting behavior in high mercury individuals" in this report).

d. Post-hatching Period: Behavioral relationships with Hg risk

Once chicks hatched, TABs were conducted on the entire loon family by 2-4 observers. Because loon chicks molt into their next downy stage at approximately two weeks and retain downy feathers over half of their body until 5 ½ weeks of age, we separately investigated the behavior of adults according to these time periods. This is consistent with Nocera and Taylor (1998) and Counard (2001), who recognized 1-12 (downy young, or DY) and 13-40 (small young, or SY) chick age periods in their studies.

Figure 11. Average percent of time spent by adult Common Loons (M & F) during post-nesting, tending 1-12 day old chicks in 5 Hg exposure categories.

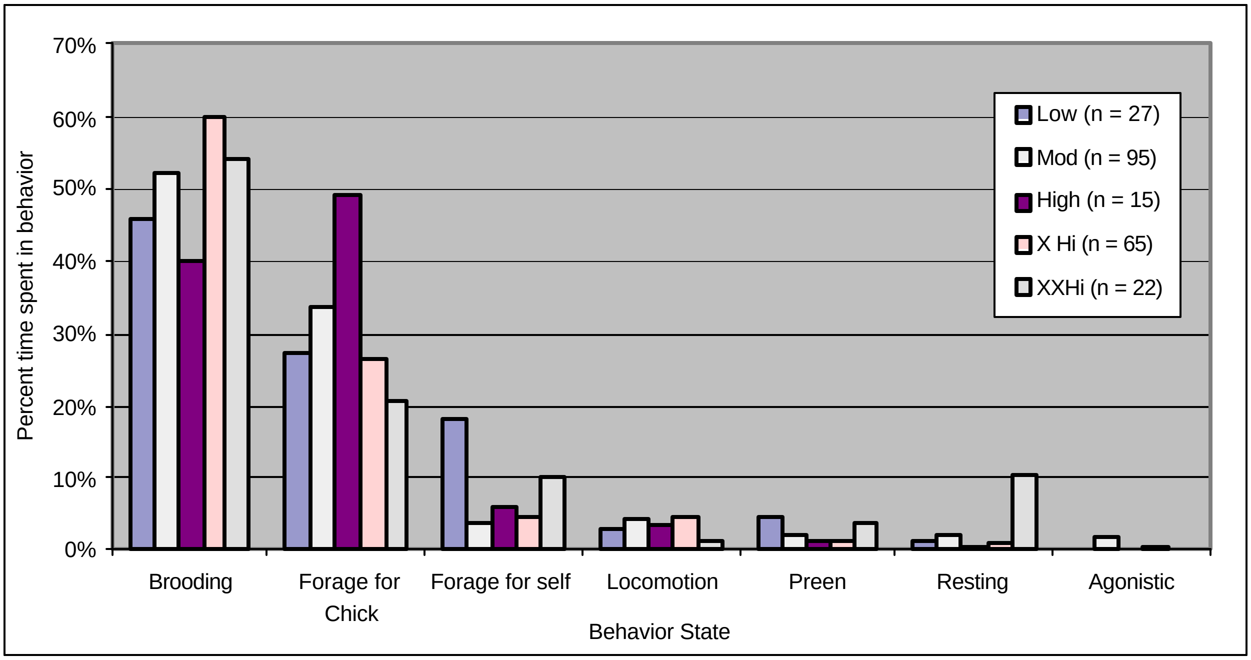
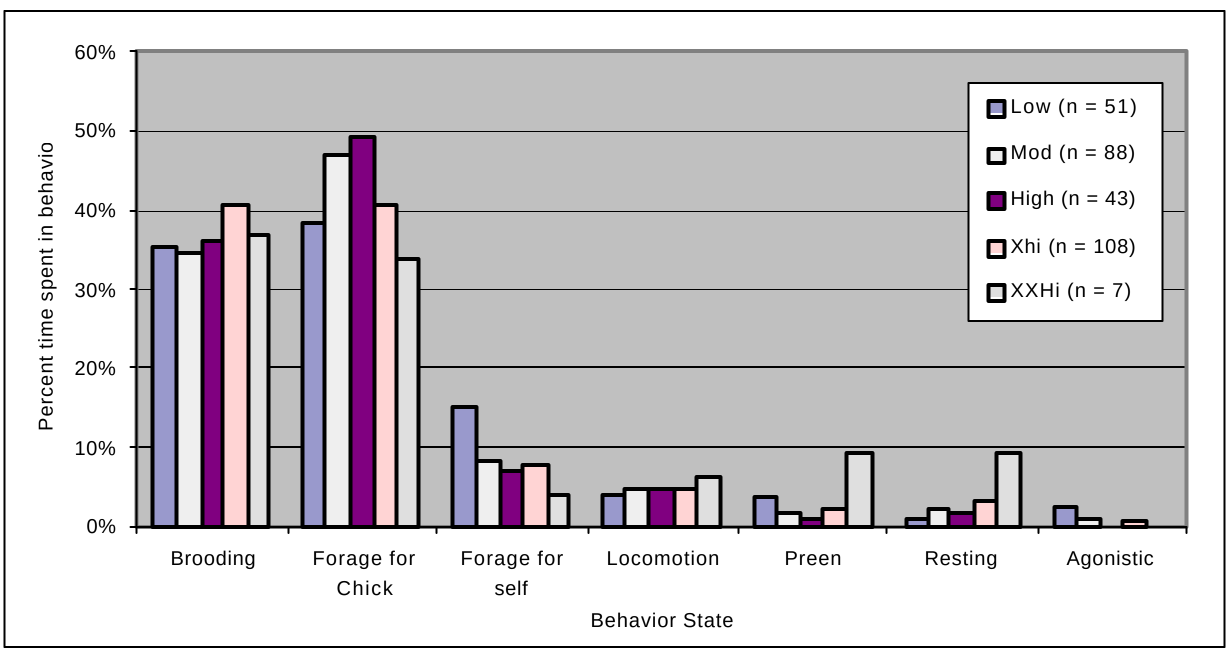


Figure 12. Average percent of time spent by adult Common Loons (M & F) during post-nesting, tending 13-40 day old chicks in 5 Hg exposure categories.



Post-hatching Period: brooding

Adults in the 1-12 age period spent the highest percentage of their time brooding (40-60%), while adults in the 13-40 age period appeared to shift previous time spent brooding (35-40%) towards foraging for chicks. Although the number of hours spent for the high category are low and therefore may misinterpret the actual time spent by high risk adult loons, time spent brooding 1-12 and 13-40 day-old chicks showed an increasing trend as Hg levels increased (Figures 11 and 12). Linear regression of this data found a significant positive relationship between the percentage of time male and female loons spent brooding 1-12 day-old chicks and blood Hg levels (R2 = 0.312, p = 0.037; Figure 13). The percent of time both adults spent brooding was not significantly correlated with blood Hg in the 13-40 period (p = 0.463), presumably because of the shift in time spent to other behaviors such as foraging for chick. The correlation between percent of time male and female loons spent brooding 1-40 day old chicks was marginally significant (R2 = 0.177, p = 0.072). We also measured 5 subcategories within brooding behavior itself (locomotion, drift, preen, underwing, and on-back), and found adults spent significantly less time locomoting while in brooding behavior than low Hg risk loons ($p<0.05$).

As this and other studies such as Bouton et al. (1999) seem to indicate, gender is often an important and significant factor in relation to the effects of mercury on behavior. In most cases, males are reported to be more significantly impacted by mercury in behaviors such as foraging than females, due to males' tendency to take larger fish (and forage more) and females' ability to depurate Hg into eggs. We found the relationship between the percent time females spent brooding and female blood Hg to be marginally significant for the 1-12 period (R2 = 0.309, p = 0.075), and the 1-40 periods (R2 = 0.217, p = 0.059; Figure 14). This relationship was not significant for males in the 0-40 period (p = 0.603), and it was not calculable for the 1-12 period due to sample size. These findings are consistent with slight gender-brooding differences found other studies, (Mager (1995), Evers (1994). Although gender-specific regressions in our study are limited by sample size, we believe that our general findings of relationships between time spent brooding and blood mercury are important.

Figure 13. Average time adult male and female loons spent brooding 1-12 day-old chicks related to their mean blood Hg levels in Maine, 1998-00.

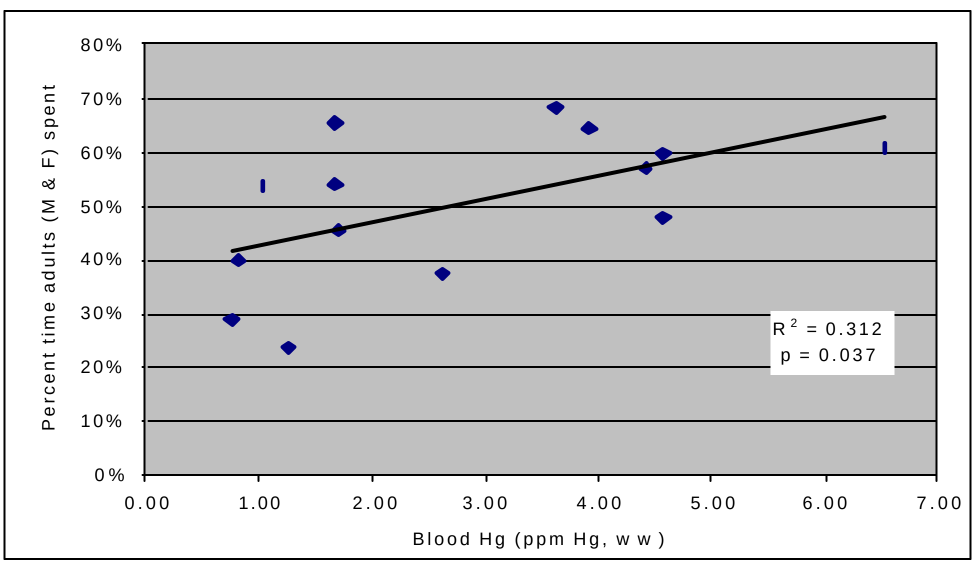
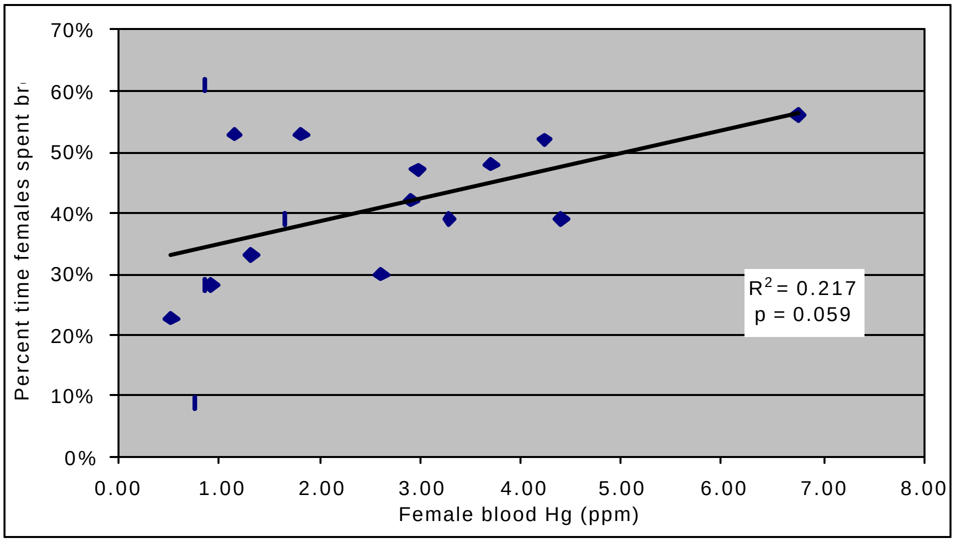


Figure 14. Average time female loons spent brooding 1-40 day-old chicks related to female mean blood Hg levels in Maine, 1998-00.



Post-hatching Period: foraging (for chick and self)

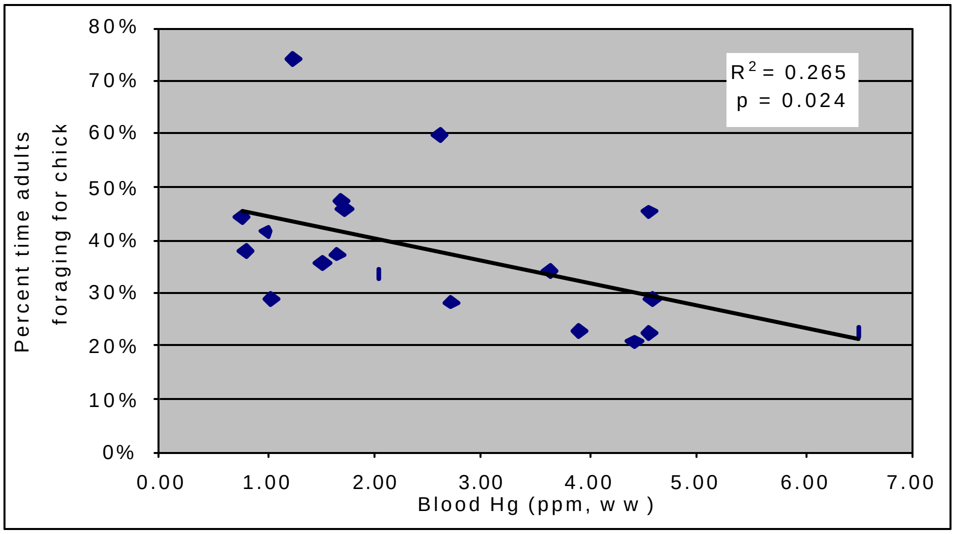
Time adults spent foraging for chick in both the 0-12 and 13-40 periods increased in a step-wise pattern from low to high, but then declined for the highest level (Figure 11 and Figure 12). This year's addition of the XXHi category continued this trend, XXHi individuals spent less time foraging for chicks than those in the XHi category. The patterns seen in the percent time spent foraging for chicks across risk categories in the 1-12 and the 13-40 periods are virtually identical suggesting that chick age is not a confounder in the relationship. Linear regression analysis for data in both age groups reveals a significant negative correlation between percent time adults spend foraging for chick and blood Hg (R2 = 0.265, p = 0.024; Figure 15).

Adults in low risk categories spent the most time foraging for themselves out of all risk categories [18% for the 0-12 period (Figure 11), and 15% in 13-40 period (Figure 12)], which is similar to post-nesting levels in Michigan (Evers 1994). Percentage of time adults spent foraging for self in the other risk categories ranged from 4-10% in the 1-12 period and 4-8% in the 13-40 period. Although a both periods may tend toward decreasing time spent in foraging for self behavior, the downward "stepwise" pattern between risk categories is most evident in the 13-40 period.

Our findings regarding Hg-foraging behavior relationships agree with those reported in the literature. Bouton et al. (1999) reported significant relationships between mercury and motivation of low dosed (0.5mg/kg) juvenile Great Egrets to hunt prey, as well as an increased mean time necessary to capture fish and decreased tendency to eat prey items. Counard (2001) reported a significant relationship between chick begging and mercury, which may be related to a compromise in the adults' ability to adequately meet energetic demands of chicks while foraging. Mercury has also been implicated in impairing learning and physical abilities (Inouye et al. (1985) and Burbacher (1990) in Bouton et al. 1999), as well as reduced motor skills due to damage to the cerebellum and cerebrum (Wolfe (1998). Frederick et al. (1997) reported on preliminary data from a dosing study that suggests a debilitating effect of Hg on vision in birds. Frederick et al. (1997) cites other studies that have linked alterations in photoreceptor function with mercury (Fox and Sillman 1979, Gitter et al. 1988). We have further explored foraging-Hg relationships in this report, using the rate of chick-feed

(Figure 17) and dive events.

Figure 15. Average time adult male and female loons spent foraging for chicks while brooding 1-40 day-old chicks related to their mean blood Hg levels in Maine 1998-00.



Post-hatching Period: Preening and Resting

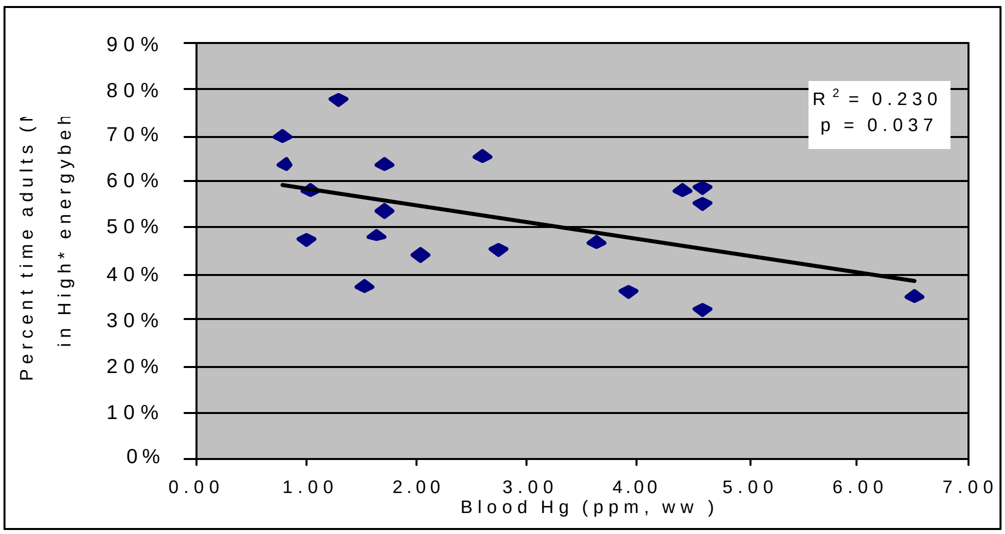
We quantified all self-maintenance behaviors such as preening and bathing in our field studies. Time spent preening in Michigan was consistently 4-5%, however time spent preening in Maine loons was significantly lower ($p<0.05$), with only 1% time spent for high and extra high Hg risk categories. In general, adults in higher risk categories tended to spend less time in these self-maintenance behaviors such as and preening and foraging for self. Both Nocera and Taylor (1998) and Counard (2001) have found significant positive relationships between mercury and preening in juvenile loons. Although once thought that this increase could be related to a decrease in back riding, Counard (2001) argues that this was not the case in her study. Although our behavior data on adults and juveniles does not display the same relationship, it is worth noting the significant increase in the percent time spent preening by XXHi adult loons in the 13-40 period (Figure 12). Bouton et al. (1999) reported a higher frequency of preening in dosed Great Egrets. Heinz (1974) reported a relationship between mercury and fright stimulus, which, as Counard (2001) suggests, may be related to increases in preening in high risk individuals.

Perhaps most notable is the significant difference in the percentage of time adults in the XXHi category spent in resting behavior in comparison to all other categories (Figures 12 and 13). Despite the small sample size, this is still interesting given the stepwise pattern of male resting behavior displayed in Figure 10. Time spent in this category is the epitome of minimal parental care. It also represents a measurable shift of time spent to the lowest energetically demanding behaviors. This shift would be of significant concern if it alludes to a deficiency in other important behavior states such as foraging for chick (Figure15) and self as our data suggests. Because of similar findings in other studies relating mercury to decreased activity levels and lethargy (Bouton et al. (1999, Thompson 1996, Heinz 1996), we have recategorized our data to further explore this relationship.

e. Behavioral Relationships: mercury and energy expenditure

As previously mentioned in discussions of foraging, other studies have reported relationships between mercury and decreased activity levels (increased lethargy), motivation to hunt and thermoregulation (Bouton et al. 1999, Thompson 1996, Heinz 1996). Our behavioral findings point towards a negative relationship between blood mercury and/or risk category and high-energy behaviors such as foraging. Conversely, the percentage of time adults spent in behaviors associated with lower energy demands (such as brooding, and resting) appeared to increase with blood mercury and/or exposure category. To further address this tendency, we separated all behaviors of adults brooding 1-40 day-old young into these two categories based on our current perception of their energetic demands. Foraging for chick, foraging for self, locomotion, preening and agonistic behaviors were grouped together in the "high" category, while brooding and resting (the sum of drift and sleeping) behaviors were

Figure 16. Average time adult male and female loons spent in high energy behaviors while brooding 1-40 day-old chicks related to mean blood Hg levels in Maine 1998-00.

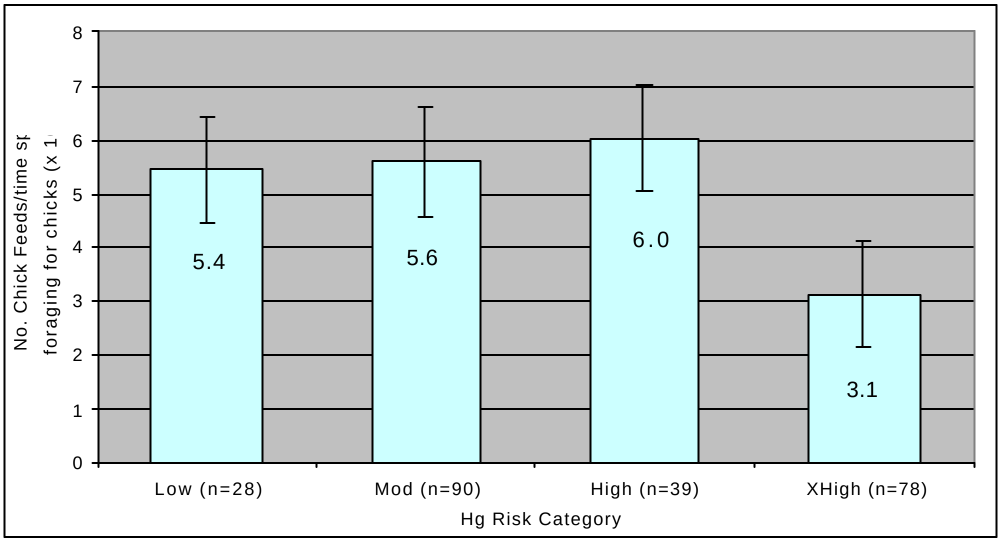


categorized as low. We found a significant negative relationship between mercury and the percent time adult male and female loons spent in high energy behaviors while brooding 1-40 day old young (R^2= 0.230, p = 0.031; Figure 16). Our findings are very similar to those reported by Bouton et al. (1999), who reported a general tendency for dosed Great Egrets to spend less time walking, pecking and flying than controls. More important, they similarly report a "negative relationship between mercury dose and the amount of time spent in active, energetic, and maintenance behaviors." We believe that this concept may be the underlying explanation for many of the relationships discovered among specific behaviors and mercury in this study.

f. Adult Behavior Event Analysis

Few studies have used behavior events to give insight into behavioral impacts of Hg on Common Loons (Counard 2001, Nocera and Taylor 1998). These counted behaviors are easily defined and quantified in the field, thereby minimizing observer and sampling biases (Appendix 1). We believe that some behavior events can give us helpful insights into subtle behavioral differences between risk categories.

Figure 17. Comparison of average chick feeding event behavior by male and female Common Loons brooding 13-40 day old chicks in 4 Hg exposure categories.



Chick Feed Events

The act of feeding young is perhaps one of the most important components of parental care. This is especially true in the first eight weeks after hatching, when the young are most dependent on the adults for food (Barr 1986). Figure17 displays our analysis of chick-feeding events by adult male and female loons brooding 13-40 day old chicks in four Hg risk categories (We did not find a similar pattern for the 0-12 age group). The quantification, therefore, is a rate of chick feeding during the "foraging for chick" behavior state. Although adults in the extra high category in Figure 15 appeared to feed their chicks at a rate that was 42.5% lower than controls, the relationship between low and xhi risk categories was not significant ($p = 0.108$, ANOVA, single factor). This difference may be related to the small sample size in the low category.

Dive Events

Olsen et al. (in prep.) used adult dive event data from this study to address potential impacts of mercury on foraging behavior. Non-parametric testing found a significant difference among diving frequencies of Common Loons of various exposure levels ($H = 8.75$, $df = 3$, $p = 0.033$), and a positive correlation between the diving frequency and mercury risk ($r = 0.136$, $N = 249$, $p = 0.032$). Olsen et al. suggest that since mercury is known to inhibit heme (Marks 1985), it lowers the oxygen carrying capacity of the blood, thereby limiting the duration of dives, increasing the frequency of dives necessary to meet caloric needs of themselves and their young. Counard (2001) reported a negative relationship between time loon chicks spent diving and Hg (p =

0.009, R2 = 0.32). Other studies such as Bouton et al. (1999) presented significant findings of a relationship between mercury and the mean time necessary to capture fish. Dosed Great Egrets (0.5mg methyl HgCl/kg) were found to be consistently slower at capturing fish than controls (p = 0.041). Significant effects of age and sex were also reported (p = 0.004, and p = 0.004, respectively), and dosed birds were significantly less likely to eat fish presented to them (even in cases where they caught them) in both camouflage (p = 0.0003) and contrasting (p = 0.003) pool background settings. Findings in this and other studies suggest that mercury may interfere with foraging behavior, vision, and coordination.

g. Adult Behavior Summary

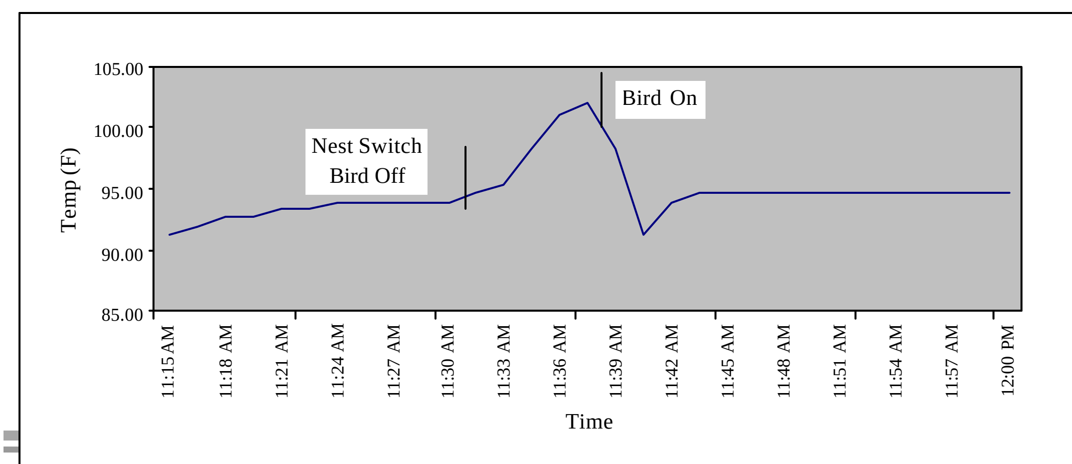
The analysis of 1998-2000 adult loon behavior data indicates a relationship between Hg and several aspects of loon behavior. Stepwise patterns were observed in several behavior categories among loons in Hg risk categories in all nesting stages. Regression analysis on several of these behaviors revealed statistically significant relationships between blood mercury and behavior. The results of this study indicate a relationship between high Hg risk situations and (1) less time spent nest sitting, (2) specific cases of abnormal incubation behavior patterns, (3) less time spent foraging for young and themselves, (4) increasing time spent brooding and resting, (5) increased dive frequency while foraging, (6) behavior impacts biased towards males.

These findings reveal an underlying tendency for mercury to negatively impact the amount of time adult loons spend in high-energy behaviors, thereby shifting time spent into behaviors with lower energetic demands. These behavior alterations could be the precursor of decreasing reproductive performance, chick survival and adult survival.

h. Temperature Dataloggers as measures of adult incubating behavior

Because of known incidences of high Hg risk males exhibiting abnormal incubation patterns, we have attempted to fully measure time spent over several consecutive 24-hour periods. Temperature data loggers were placed in 12 nests during 1998, for a total of over 1,600 hours of monitoring and in four different nests in 1999 for a total of 400 hours. Both nest and ambient temperatures were monitored on 8 of the 12 sites in 1998 and all sites in 1999. A clear example of nest switching as indicated by the data loggers occurred on the Chain of Ponds – Upper territory on June 24 (Figure 7). The nest temperature rose after the loon left the nest, as the nest was in direct sunlight. The nest temperature then declined seven minutes later due to the water and increased aeration of egg turning associated with the arrival of the loon's mate.

18. Nest temperature (F) for a Common Loon pair, June 24, 1998.



An average of the daily nest temperature (7 am to 10 pm) of 8 data sites was 81.04° F, with a correlative average ambient temperature of 67.56° F. Average nighttime (10 pm to 7 am) nest temperature was 77.35° F, with a correlative ambient temperature of 59.16° F.

While no significant differences between high-level mercury lakes (as determined via the loon risk to mercury exposure) and low-level mercury lakes were detected, several nights recorded on moderate to high-level mercury lakes showed inverse relationships between ambient and nest temperatures. These relationships might indicate possible high-stress periods and need to be further investigated. An analysis of diurnal and nocturnal temperatures on 8 lakes indicates high-level mercury lakes may have a higher disparity of daily nest temperatures. Initial data (n=3 nests) show moderate-level mercury lakes have an average of 2.09° F difference between diurnal and nocturnal nest temperatures. High to extra-high-level mercury lakes (n=3 nests) have a daily nest temperature fluctuation of 4.43° F.

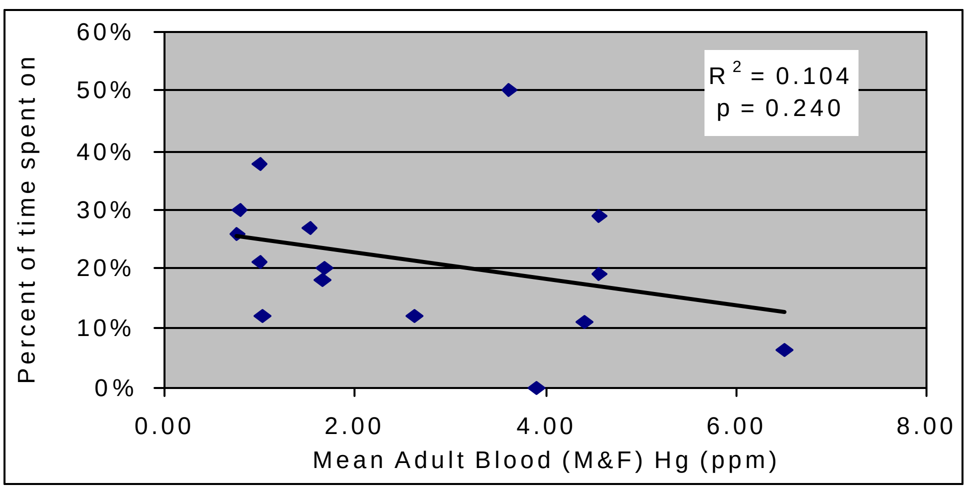
The use of these temperature data-loggers is promising and in time would provide a greater confidence and accountability of 24-hour incubation patterns by breeding pairs.

i. Juveniles: Behavioral relationships with Hg risk

Nocera and Taylor (1998) and Counard (2001) have both found significant negative correlations between blood Hg levels and back-riding in juvenile loons, and a positive correlation with preening. Nocera and Taylor (1998) postulated that chick survival might be affected by increased rates of predation as a result of not being on the adult's back and less time spent foraging.

We found no significant relationship ($p>0.05$) between the time downy chicks (0-12 days) spent back-riding and adult blood Hg levels (Figure 19).

Figure 19. Time spent back-riding by 0-12 day old chicks, 1998-2000.



However not significant ($p>0.05$), Figure 19 shows a negative correlation trend between downy young (0-12 days) back-riding and adult blood Hg levels. This trend could have a negative impact on juvenile survival by increasing the chick's exposure to predators (Nocera and Taylor 1998). With an increased sample size, we feel the correlation will become stronger. We also did not find a significant relationship between back-riding and adult blood Hg levels in 13-40 day old chicks ($p>0.05$).

3. Survival Relationship with Mercury

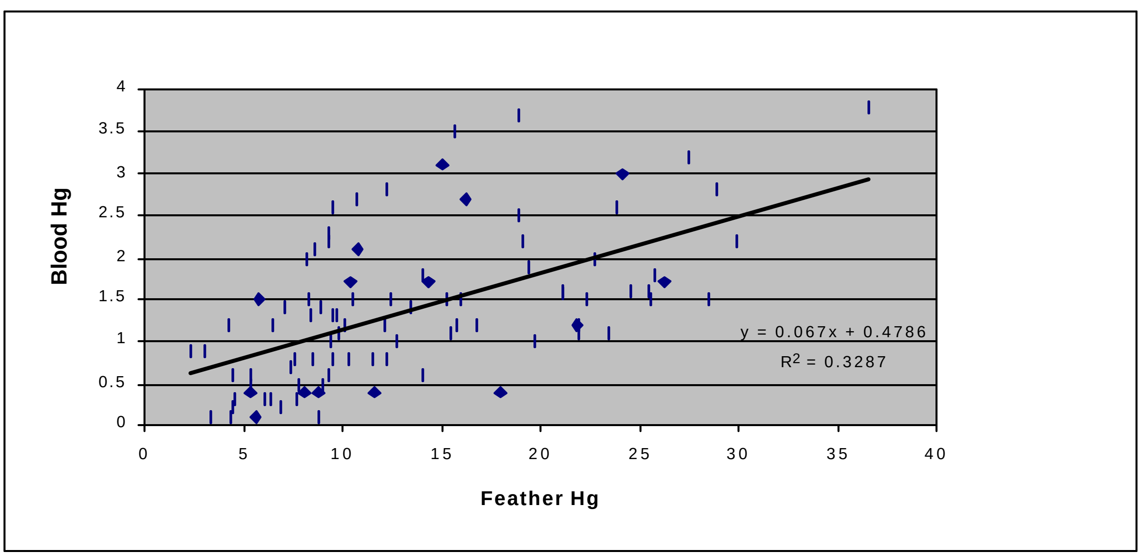
Adults and juveniles show increasing levels of Hg in their body burdens over time (Evers et al. 1998a, BRI unpubl. data). Although birds have natural defense mechanisms for depurating (e.g., feathers), demethylating (e.g., liver and kidney), and sequestering (e.g., egg) mercury (Thompson 1996), high-risk individuals accumulate more Hg than they are able to annually regulate. Excess Hg binds to protein in the muscle tissue and remobilizes during stressful events. Feather molts are energetically demanding, particularly the full remigial molts that loons experience for two weeks during the winter. Because muscle protein reservoirs are associated with feather protein (Murphy 1996), the remobilization of proteins during feather molt partly reflects the available body burden of MeHg in an individual loon.

a. Adult Loons

We found a significant amount of this muscle-bound MeHg originated from prey during the breeding season. In New England loons, there was a significantly positive relationship between blood and feather Hg levels (r^2=0.32, $p<0.01$) (Figure 20). In past studies, we did not find a significant relationship in breeding loons in the Great Lakes, Pacific Northwest, and Alaska (Evers et al. 1998a).

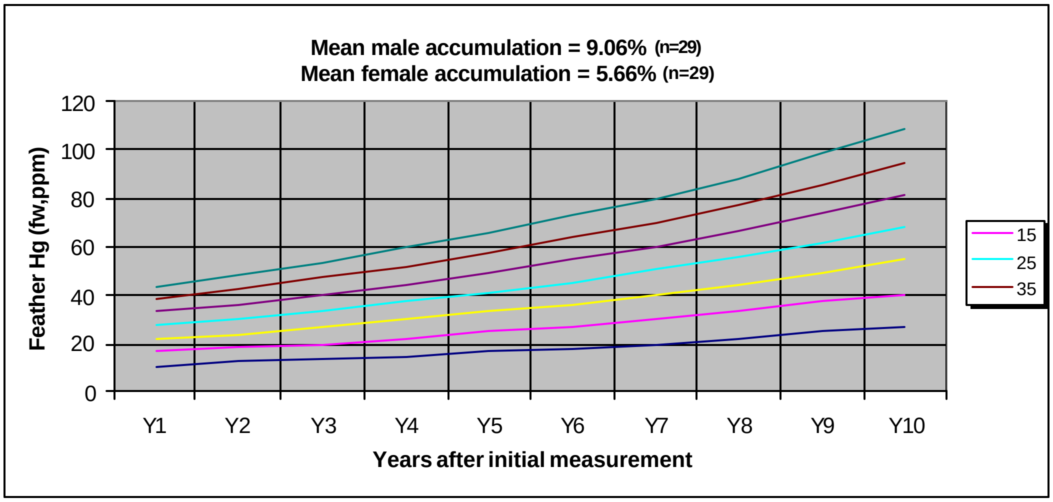
Therefore, the feathers grown during the winter and sampled during the breeding season are indicators of chronic body burdens of Hg. Feather samples collected from recaptured adult loons now indicate this bioaccumulation of Hg is measurable over time and in high-risk populations significantly increases annually. Adult loons were recaptured over the past 1-5 years from Maine lakes. Of 15 males representing 29 accumulation-years, 23 (79%) of those years showed an increase. The mean annual accumulation rate in males was 9.06%. Of 8 females that were recaptured, representing 29 accumulation-years, 21 (72%) of those years showed an increase. The mean annual accumulation rate for females was 5.6%. Male accumulation rates were most likely higher than females because of the females' ability to sequester Hg in eggs (Kambamandi-Dimou et al. 1991) and the tendency to eat smaller prey than males (Evers and Reaman 1998). The body mass of males average is 23% larger than females in New England.

Figure 20. Relationship between blood and feather Hg levels (ppm) in New England Common Loons.



The mean feather Hg levels in Maine's male loons is 14.8 +/- 8.2 ppm (n=87) and in females is 9.8 +/- 5.0 ppm (n=87). A temporal extrapolation using an accumulation rate of 9.06% for a male with 15ppm of Hg in its feather places that individual at high risk and potential impacts in 3 years (i.e., 20 ppm) and probable impacts (i.e., 35 ppm) in 7 years (Figure 21). Although female Hg bioaccumulation rates are lower ($p<0.5$) this rate still only increases the female's reproductive expectancy two years. Because loons are K-selected species, long-term impacts on their reproductive success can potentially have severe population effects.

Figure 21. Bioaccumulation of Hg measured in feathers of recaptured adult loons.

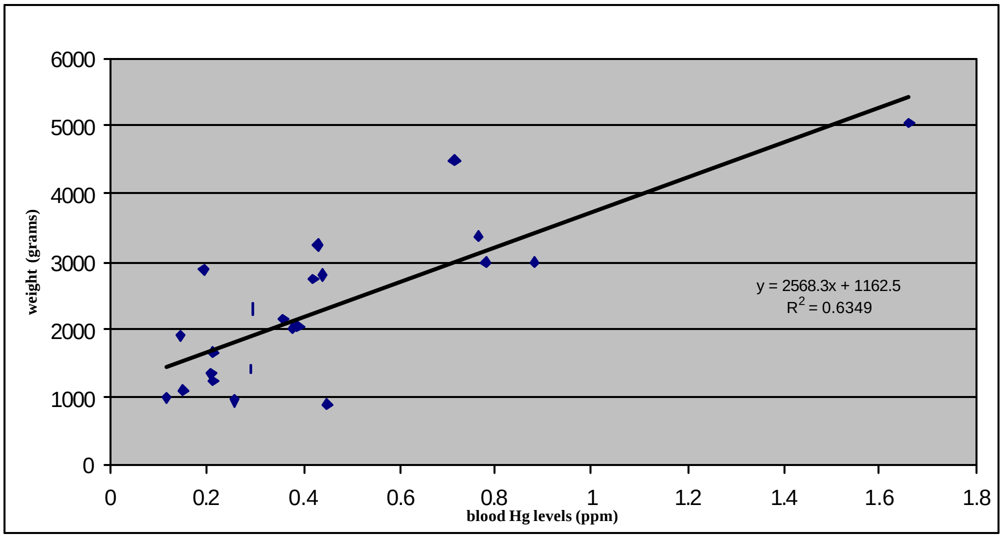


b. Juvenile Loons

Although we have shown environmental Hg levels in Maine to probably impact bird embryos, there are also potential impacts on juveniles if they should hatch into a high mercury situation. We suspect that loons in Maine have lower growth and survival rates that are related to the uptake of MeHg from their prey. Once a juvenile loon has fully grown feathers, its body burden of Hg can no longer be depurated and begins to build up in its blood, brain, organs, and muscle (Frederick et al. 1997). Young loons have three feather molts before attaining a juvenile plumage. From hatching to 12-14 days a black downy plumage covers the chick's body and that is replaced by another downy, but brown plumage lasting for 3-4 weeks. At 6 weeks many of the contour feathers have replaced the downy plumage which can only be found on the nape, neck, and flanks. Flight feathers begin to sheath at 4 weeks and are fully sheathed by 12 weeks. Rate of primary growth is consistent from 5 to 11 weeks, while overall growth rates begin to fall at 10 weeks (Barr 1996).

We captured 3 to 12 week old loons on Flagstaff and Aziscohos Lakes from 1994-99 and found a significant correlation ($p<0.01$) between blood Hg concentrations and weight on these high Hg lakes (Figure 22). Although loon weights vary with nutritional and physical stress due to sibling rivalry and habitat quality, we used weight as an indicator of age. Therefore, as young loons aged on their natal territories, Hg levels significantly increased ($p<0.01$). Juvenile loons from lakes with lower risk to MeHg availability also exhibited significant increases but were more weakly correlated with weight.

Figure 22. Blood Hg concentrations versus weight in juvenile Common Loons from Aziscohos and Flagstaff Lakes, 1994-00.



In 1999, we also recaptured four juvenile loons of known age (Table 5). Although sample size precludes conclusions, young loons do appear to have increasing blood Hg levels while maturing on their natal lakes. The rate of Hg increase in these four loons was 1.0 to 3.6% per day.

Table 5. Change in blood Hg concentrations of known-age juvenile loons recaptured 14-40 days later in Maine and New Hampshire, 1999.

Lake	Hg Risk	Change in Days	Change in Hg	Change in Weight	% Increase of Weight	% Increase of Hg	% Increase of Hg/day
Aziscohos	XH	40	0.421	2190	95%	143.2%	3.58%
Aziscohos	XH	22	0.073	1100	51%	20.3%	1.00%
Aziscohos	M	18	0.048	980	52%	32.6%	1.8%
Winnipesauke	M	14	0.101	760	38%	45.5%	3.3%

4. Reproduction Relationship with Mercury

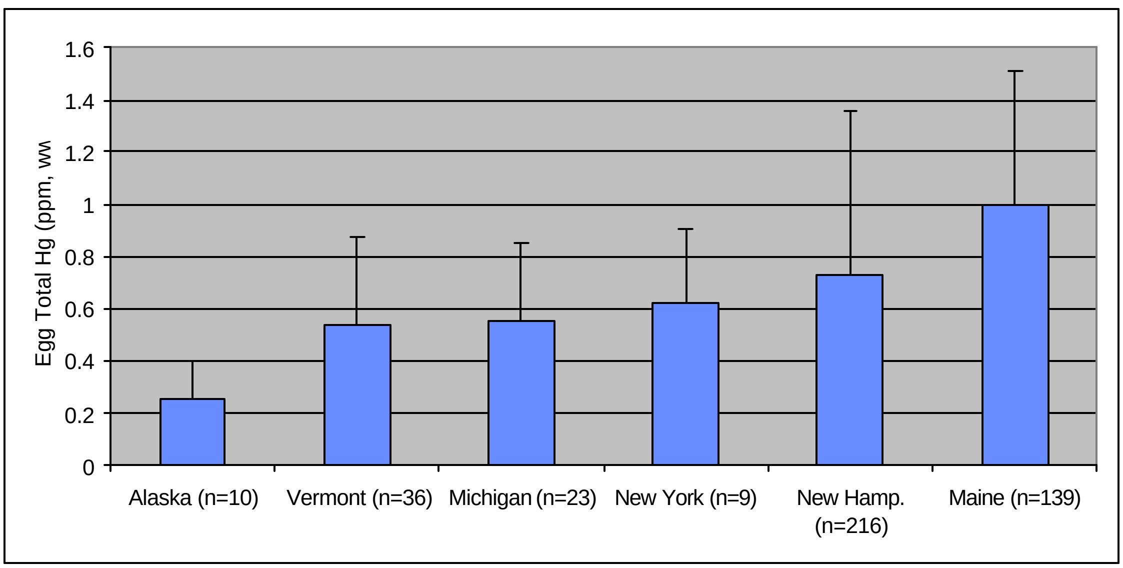
a. Egg Development and Hatching Success

Controlled studies have shown that mercury toxicity impacts egg development and hatchability at levels (i.e., 0.5-4.4 ppm) that are found in this study (Borg et al. 1969, Fimreite 1971, Heinz 1979, Spann et al. 1972, Gilbertson 1974). Lower reproductive success in birds has been documented at Hg levels lower than those that cause observable effects on adult behavior and survival (Scheuhammer 1991). Thompson (1996) summarized several controlled studies of captive birds and predicted dietary concentrations of 0.6 ppm (wet weight) (converted from 3.0 ppm dry weight) cause impaired reproduction in birds, yet have little effect on adult survival. Barr (1986) found loons laid fewer eggs when prey Hg averaged 0.3-0.4 ppm and no eggs were laid when prey averaged over 0.4 ppm of Hg. Several of our study lakes contained yellow perch between 10-15 cm that exceeded 0.3 and 0.4 ppm.

We collected morphometric and developmental information from 139 eggs that were abandoned on Maine lakes because of flooding, human disturbance, and other reasons. Loons typically lay two eggs within 24 hours of one another. Mean egg size in Maine was 145.9 +/-22.1 g (weight), 91.9 +/-3.8 mm (length), 56.9 +/- 2.4 mm (width), and 140.6 +/- 19.8 g (volume). In birds, first-laid eggs are larger than ones following. We ran a discriminate function analysis (DFA) on 21 egg clutches from Maine and New Hampshire loons for the four morphometric measurements and found length to be the best predictor variable (72% correct) of egg laying order. If DFA can predict the largest egg (in nests where one egg hatches and one does not) we can then classify egg-laying order in one-egg collections as well as two-egg clutches. This would help reduce predictive errors with one egg clutches since we have found mean Hg concentrations between eggs in a clutch to vary by 36%.

Loon egg Hg levels were related to (1) geography, (2) viability, (3) weight, and (4) within clutch variability. Maine loons had egg Hg concentrations with a mean of 0.76 +/- 0.12 and ranged from 0.36 to 2.65 ppm (wet weight). Except New Hampshire, mean egg Hg concentrations were significantly higher ($p<0.05$) than other North American study sites in Alaska, Montana, Minnesota, Michigan, New York, and Vermont (Figure 23). New Hampshire mean egg Hg concentrations tended to be lower than Maine's.

Figure 23. Mean Hg concentrations in Common Loon eggs from selected sites, 1993-00.



We did not find egg Hg to significantly differ between fertile and infertile eggs ($p>0.05$). Although Spann et al. (1972) found lower survival during the third trimester of pheasant embryos, loon egg Hg concentrations did not significantly differ between five developmental stage categories ($p>0.05$). However, since over 88% of Maine's loon eggs have Hg concentrations at levels that other studies have shown impact, we plan to develop a DFA that can better predict egg laying order for nests that have two-egg clutches and reduce confounding variables.

We found that egg weights had a marginally significant tendency to decrease as Hg levels increased ($p=0.09$). Because egg weight is closely related to an individual's body weight (Rahn et al. 1975) we analyzed the weights of adults recaptured in New England over time. We compared the change of weight in adults recaptured over one year later and found that those rated with an extra high (n=17) and high (4) risk had a decline in body weight, -1.1% and –1.2% per year, respectively. The decline for the extra high recaptured adults was significant (p=0.03, n=16 years). Those recaptured adults with a moderate (n=8) and low (n=6) risk did not have a significant change in body weight (0.15 and 0.55% per year, respectively) (p>0.05).

Analysis of 32 complete clutches revealed that 85% of the larger sibling egg had higher Hg levels. Clutches with at least one egg over one ppm of Hg (i.e., clutch with a high or extra high risk to Hg toxicity) had a within-clutch weight difference of 12.3 grams (n=14) while clutches with both eggs under 0.5 ppm of Hg (i.e., clutch with a low risk to Hg toxicity) had a significantly less within clutch weight difference of 6.9 g (n=18). Larger sample sizes are needed to substantiate these findings.

We conclude that territorial adult loons are losing weight over time and that loss of body mass is reflected in egg weights. Whether this is related to Hg body burdens reducing fitness or other factors such as ageing needs to be further explored. Additionally, egg weights within a clutch appear to be impacted by Hg with the larger egg having a higher probability of Hg toxicity than the smaller egg.

b. Impacts on Overall Productivity

Barr (1986) found a strong negative correlation between the successful use of a territory by breeding pairs and Hg contamination (measured in forage fish). Loon reproduction, capacity to lay eggs and maintain nest/territory fidelity, was impacted with fish Hg levels of 0.3 ppm and no reproduction occurred in areas with fish Hg levels of 0.4 ppm. Burgess et al. (1998) also found a significant negative correlation between loon blood Hg levels and reproductive success by using the ratio of nesting vs. territorial pairs (i.e., detects whether an egg was laid). Weaker but negative correlations were also found with hatched young per nesting pair and fledged young per territorial pair. Like the high-risk Hg sites in Kejimkujik NP (Burgess et al. 1998), we found a significant correlation between some of the reproductive success ratios and adult blood, juvenile blood, and egg Hg concentrations (p<0.05). Variation and the lack of agreement between the various reproductive success ratios was likely related to confounding variables including predation, density-dependent pressures, human disturbance, storm events, and other mercury-independent factors. Burgess et al. (1998) was able to statistically account for these variables and showed a significant inverse relationship with increasing adult blood Hg levels and decreasing reproductive success.

<u>*Egg Laying Success*</u>: We found similar inverse relationships with increasing adult blood Hg levels and decreasing reproductive success based on 223 loon territories representing 748 territory-years surveyed (Table 1). The reproductive measure for determining egg-laying success (nesting pair/territorial pair) was similar for low, moderate, and high-risk territories but did exhibit a significant decline (p<0.05) of 13% for the territories at highest risk to Hg (Table 6). Small fish Hg levels on many of the extra high-risk lakes had levels that exceeded the 0.30 ppm concentrations that Barr (1986) related to population level impacts.

<u>*Egg Hatching Success*</u>: How successful the pairs are in hatching their eggs are measured by the reproductive measures, hatching per territorial and hatching per nesting pair. Both of these measures exhibited significant step-down declines in hatching success from low to extra-high risk territories (p<0.05) (Table 6). Territorial pairs with extra-high blood Hg levels hatched 30% fewer young than pairs with low Hg risk (<1 ppm). Success by nesting pairs followed a similar, but less exaggerated pattern with a reduction of 19% hatched young from low to extra high risk categories (Table 6).

<u>*Chick survival success*</u>: Although young loons are rarely confirmed as having actually fledged from

their natal lake, the mortality of greater than 6-week-old-young is minimal. Therefore, most monitoring efforts use "large young" as analogous to rates of fledging. We have also used these protocols and found that the survival of young showed a tendency to decline with increasing Hg levels (i.e., 11% decrease in chick survival from low risk to extra high risk). However, because Hg levels in young loons increase as they mature (Figure 22, Table 5) they may be at greatest risk to Hg toxicity just before fledging. Young loons completely molt their flight feathers in by 11 weeks (Barr 1996) and after that have reduced pathways available to depurate the increasing amount of ingested Hg. Therefore, the period between complete remigial molt and actual fledging from the natal lake may be the time of greatest risk.

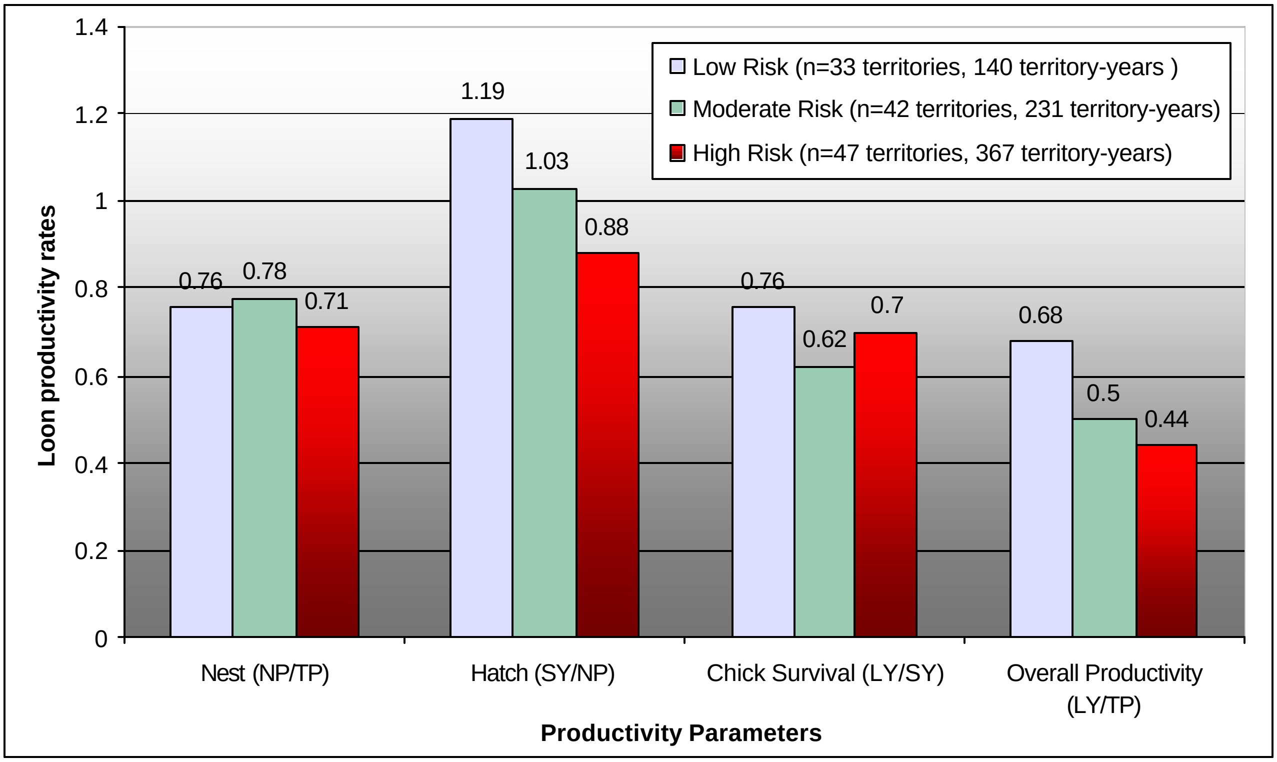
Table 6. Reproductive success of Common Loons in the Rangeley Lakes Region, Maine and selected lakes in New Hampshire.

Level of risk by territory	Low Risk	Mod Risk	High Risk	X-high Risk	Percent change Low to XHigh
Sample size: # of territories	33	42	26	21	
Sample size: # of territory-years	140	231	190	177	
Nesting Pair/Territorial Pair Ratio	76%	78%	.76%	66%	13%
Hatching/Territorial Pair Ratio	0.90	0.80	0.63	0.63	30%
Hatching/Nesting Pair Ratio	1.19	1.03	0.83	0.96	19%
Fledging/Territorial Pair Ratio	0.68	0.50	0.45	0.43	37%
Fledging/Nesting Pair Ratio	0.90	0.64	0.60	0.65	28%
Fledged/Hatched Young Ratio	76%	62%	72%	68%	11%

Overall reproductive success: We also compared fledging rates with territorial and nesting pairs and found patterns similar to hatching ratios (Figure 24). Low risk territorial pairs were 37% more successful in fledging young than high and extra high-risk territorial pairs (Table 6). The fledging/nesting pair ratio exhibited a similar trend. The number of fledged young per territorial pair is one of the more comparable numbers with other monitoring programs.

The implications of long-term declines in these reproductive measures are important to address before impacts are perceived in the loss of territorial pairs. New England breeding loon populations (before nesting is initiated) are typically comprised of 54% nesting individuals, 26% territorial but non-nesting individuals, and 20% of non-territorial individuals that are searching for opportunities to attain territorial status (Taylor and Vogel 2000). The number of non-territorial individuals represents the "buffer" in the breeding population and these are the individuals that immediately fill gaps in established territories. A noticeable decline in the number of loon territories that would be detected by traditional surveys would not happen until this "buffer" population was exhausted. If a breeding loon population reached a point where territorial pairs started to disappear, the occasional catastrophic events on the wintering area could have a long-term and substantial impact. Because the average first-year breeding age is 7 years (Evers et al. 2000) and loons typically only fledge 0.52 young per territorial pair (Taylor and Vogel 2000) recovery would be slow. Monitoring marked individuals and modeling their demographics should be initiated to avoid crisis scenarios.

Figure 24. Summary of change in reproductive measures between the 4 Hg risk categories*.



* Territorial Pair = TP, Nesting Pair = NP, Small Young = SY, Large Young (fledged) = LY

C. Risk Characterization

BRI has measured body burdens of Hg levels in Maine loons and their prey since 1994 in collaboration with members of the Northeast Loon Study Working Group. Cooperators include the U.S. Fish and Wildlife Service, U.S. Environmental Protection Agency, Maine Department of Environmental Protection, Loon Preservation Committee, Maine Audubon Society, Tufts University, and FPL Energy Maine Hydro. Because of these extensive and intensive efforts we were able to access large databases on Hg exposure in fish and loon egg, blood, and feather matrices that are relevant to this report's hazard assessment.

1. Basis for current established risk categories

Samples collected from lakes in other New England states, the Great Lakes region, and the Canadian Maritimes, were used in regional comparisons and for measuring some Hg effects endpoints. We categorized loon territories on single and multi-territorial lakes according to known exposure to MeHg (indicated by blood or eggs). The four risk categories were based on literature and *in situ* studies by the authors and their collaborators (Table 7). Low risk indicates background Hg levels that are minimally impacted by anthropogenic inputs. Loon territories that are in the moderate risk category have elevated MeHg levels but their impact levels on individuals is unknown. Loons that are in the high-risk category are exposed to toxic levels of environmental

Hg that potentially have molecular, organism, and/or population effects. The extra high Hg category is based on known impacts on loons and other birds.

Table 7. Risk categories for MeHg (ppm) availability in the Common Loon.

Matrix	Type	Low	Moderate	High	X High	Reference Base
Egg	ww	0-0.5	0.5-1.0	1.0-2.0	>2.0	Barr 1986
Blood-Adult	ww	0-1.0	1.0-3.0	3.0-4.0	>4.0	BRI[1], inferred by Barr 1986 [2]
Blood-Juv.	ww	0-0.1	0.1-0.3	0.3-0.4	>0.4	Meyer et al. 1998[3]
Feather	fw	0-9	9-20	20-35	>35	Thompson 1996, BRI[1]
Prey Fish	ww	0-0.1	0.1-0.3	0.3-0.4	>0.4	Barr 1986, Evers and Reaman 1998

[1] BRI refers to unpublished data by BioDiversity Research Institute

[2] Adult blood Hg levels are generally 10x higher than prey Hg levels (Evers and Reaman 1998) and Barr 1986 found lower reproduction of loons with prey Hg levels of 0.3 ppm and no reproduction at 0.4 ppm.

[3] Applies to 3-5 week-old juveniles, only.

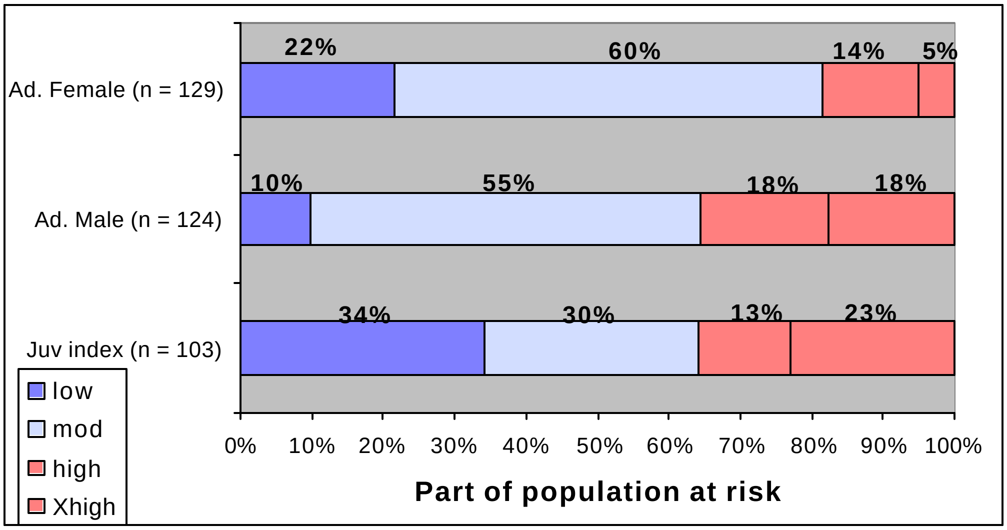
2. Common Loon Risk Profile

The following exposure assessment is somewhat based on risk categories from literature reviews (Table 7) but primarily follows this study's hazard assessment. We have high confidence in adult blood categories based on our findings with corticosterone levels and their step-wise and significant relationship with blood Hg levels (Figure 8). Adult and juvenile blood Hg categories also agreed with associated prey fish Hg levels and compared well to levels found in areas used by Barr (1986) and Meyer et al. (1998). Feather and egg Hg categories are based on extensive literature reviews summarized by Thompson (1996).

While this "bright line" or point estimate approach to risk does not integrate the actual mechanisms of how Hg impacts wildlife, they do provide an initial approach to understanding the extent of risk. The category limitations should be viewed as mean levels of potential impact. For now, the upper limit of the low risk category is the no observed adverse effect level (NOAEL) and the lower limit of the high risk category is the lowest observed adverse effect level (LOAEL). Future analysis will incorporate more statistically robust approaches that investigate the probability of the risk to the target population (i.e., Rumbold et al. 1999, Sample and Suter 1999).

Adult male loons (36%) have greater risks to MeHg availability in their prey than female loons (19%) (Figure 25). Other geographic areas outside Maine also have significant differences between

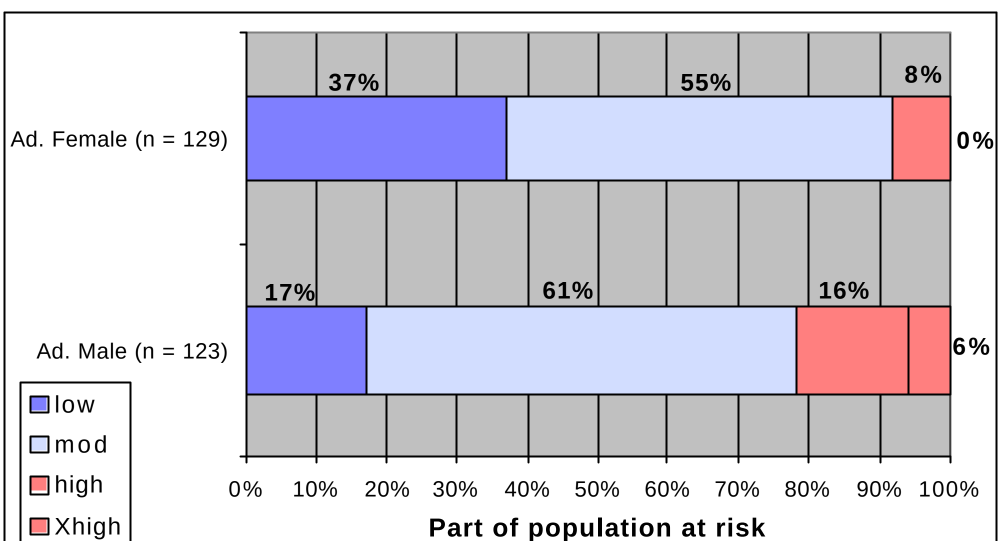
Figure 25. Exposure profile for adult and juvenile loon blood in four Hg risk categories in Maine, 1994-00.



gender Hg levels (Evers et al. 1998a). Individual Maine male loons are at greater risk than females because of their tendency to eat larger fish with higher levels of Hg and the female's ability to depurate some of her Hg body burden through eggs. Because we capture juvenile loons at different ages and we know they increase in Hg levels through the summer (Figure 22) we indexed juvenile Hg levels by weight (as an indicator of age). A total of 34% of Maine's juvenile loon population is at risk.

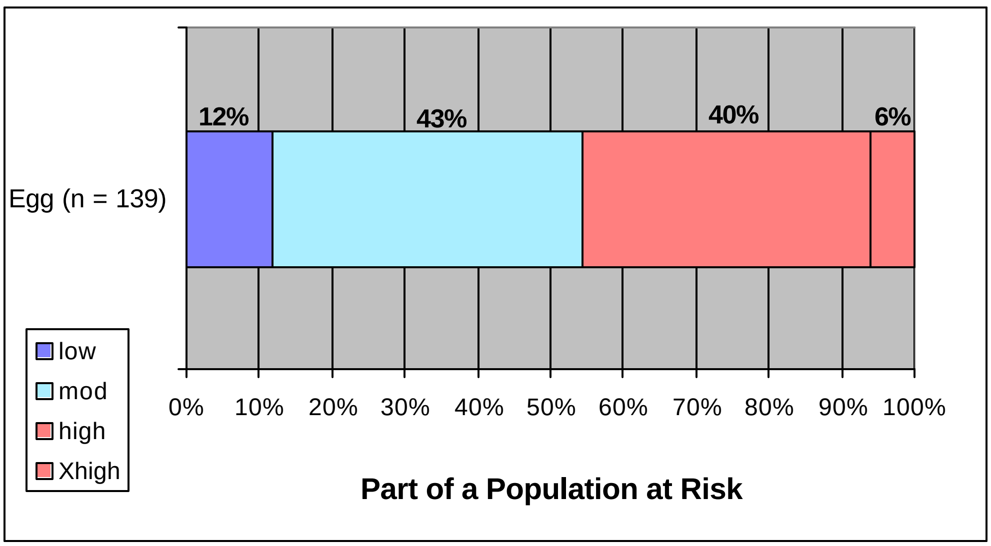
Feathers provide insight into risk to individuals and incorporate age dependent factors. There is a significant gender difference ($p<0.05$) in Maine as well as across the country. Upwards of 22% of Maine's male loons have Hg body burdens at levels that place them at high risk to Hg toxicity (Figure 26). A total of 8% of the females are at risk. The significantly increasing annual rate of Hg uptake into the body burden (9% for males and 5.6% for females) indicates that high risk individuals will likely be replaced in the breeding population by younger individuals with a lower body burden of Hg. This Hg-induced replacement rate could conceivably impact individual performance and therefore the integrity of the breeding population.

Figure 26. Exposure profile in adult male and female loon feathers in four Hg risk categories in Maine, 1994-00.



According to risk levels set by several studies on loons and other species, 46% of the eggs laid by Maine loons are at high risk to Hg toxicity (Figure 27). Although some species of birds can apparently tolerate much higher levels of Hg in the eggs without impact (e.g., Herring Gull), the loon is likely far more sensitive to Hg levels in its eggs. The extra high risk category of greater than two ppm is widely accepted as showing high mortality in many bird species (Thompson 1996). Our high-risk category is based on Barr (1986) because eggs were not hatching at his study site when they exceeded one ppm. Further studies may find that loons are able to tolerate higher egg Hg levels and we could then refine the high-risk category. Until then, this category is acceptable particularly when other birds are impacted at egg Hg levels of 0.5 ppm (Thompson 1996).

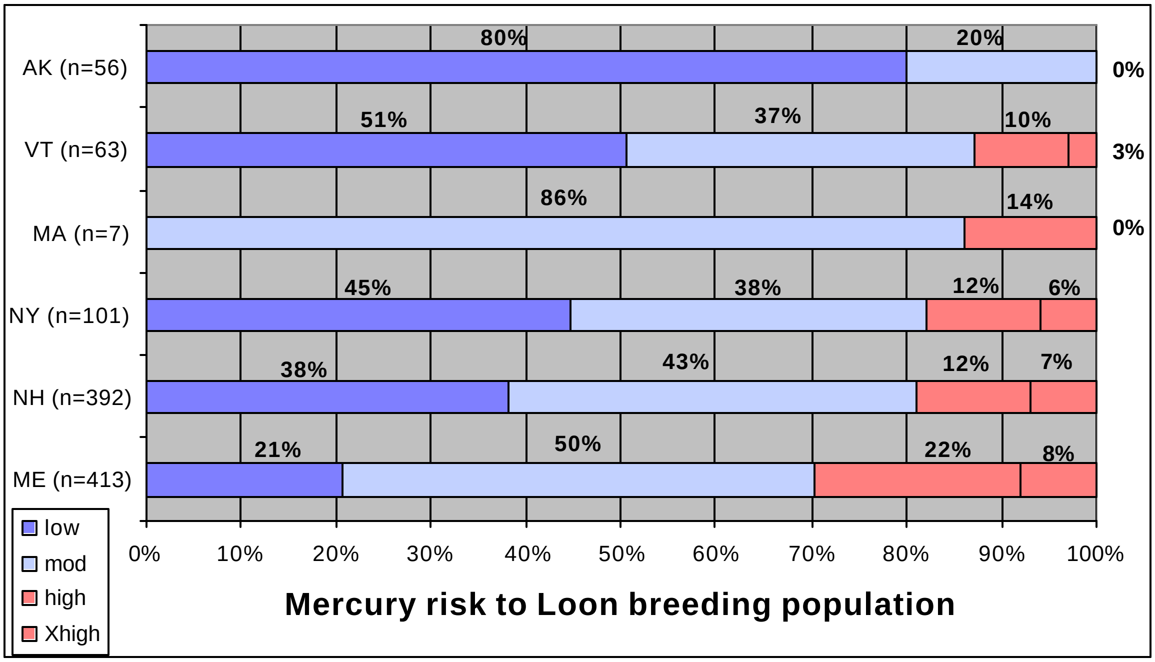
Figure 27. Exposure profile for Maine loon eggs in four Hg risk categories, 1994-00.



The cumulative risk level of Hg for loons is based on the premise that individual impacts of Hg can be determined through blood and egg measurements (Figure 28). The combination of these two matrices provides a comprehensive picture of Hg exposure that can be related to the breeding lakes. The few eggs laid by females that had blood drawn were not included here to avoid statistical problems of repeated measures. Egg Hg levels do correlate with female blood Hg levels and are therefore representative measures of the MeHg bioavailability on the breeding lake. Repetitive measures of blood Hg levels for individuals were averaged to provide one value. Feather Hg levels were not included in the cumulative risk assessment.

Figure 28. Distribution of the cumulative risks of Hg impacts for three matrices (blood, feather, and egg) in

four New England states, 1994-00.

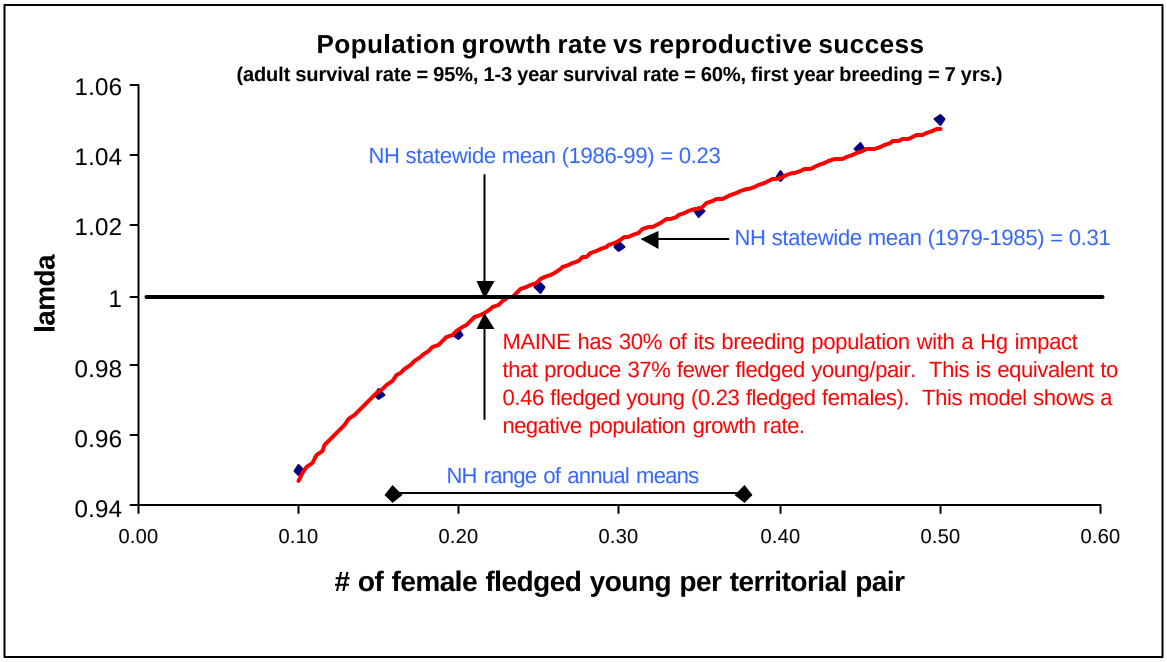


Based on opportunistic sampling strategies, current levels of Hg in Maine cause 30% of the breeding population to be at risk to behavioral, physiological, survival, or reproductive changes that are not congruent with healthy loon populations. Compared to other New England states, the risk to Maine's loons is higher. New Hampshire and New York also have "hotspots" of MeHg bioavailability that are likely related to negative population growth rates.

Although 30% of Maine's loons are at risk to impacts from Hg and these risks create a "bottom line" decline in their reproductive success of 37%, the overall impact at the population level is needed. A model was developed in collaboration with the USEPA-Office of Research and Development in Narragansett, Rhode Island to determine how the risk of Hg impacts Maine's breeding loon population (Figure 29). This model is based on the collection of three demographic parameters collected across New England and the Great Lakes (Evers et al. 2000, Evers 2001). Annual adult survival for breeding loons is 95%. The annual survival of loons at age one through three is 60%. And, the average age for the first year of breeding is 7 years. Using stage-classified matrix models a deterministic model (Caswell 2001) was developed to better understand the impacts of stressors such as Hg on the loon's breeding population.

We found that current Hg impacts are creating a negative population growth rate for Maine's Common Loon breeding population. Because our modeled results hover near lamda, refinement of this model is a high priority. Comparison of New Hampshire's loon-Hg relationship indicates similar population growth rate tendencies. Unlike Maine's loon population, monitoring efforts in New Hampshire cover the entire population and 59% of the territories have known Hg risk levels. Because it appears that Maine's loon population is at higher risk than New Hampshire's, the Maine negative population growth rate is likely a conservative estimate. Another indication of mercury's potential impact on Maine's loon population is the significant decline in New Hampshire's loon productivity from the period 1979-1985 (0.31 fledged female young) to the period 1986-1999 (0.23 fledged female young).

Figure 29. A model of the Common Loon's population growth rates based on parameters from loons across New England the Great Lakes. Comparison of the current impacts of Hg on loons indicates a negative population growth rate.



RECOMMENDATIONS

The weight of evidence strongly suggests impact of Hg on a significant component of the breeding loon population and likely other piscivorous wildlife in Maine. This information base begins to address two legislative strategies. First, in the 1997 Annual Report, The Land and Water Resources Council submitted an evaluation and recommendations to the Joint Standing Committee on Natural Resources on 28 January 1998 (LWRC 1998). In strategy 9, in regard to focusing on "biological research on the effects of mercury on the health of loons, fish, and other wildlife with elevated mercury levels," the Council proposed the action to "include projects aimed at documenting any reproductive or other effects which may be associated with elevated mercury levels." This was to be in partnership and funded by the second legislative-directed program—the Surface Water Ambient Toxics program (SWAT). In a 1995 technical report, SWAT (1997) included as their goals that "Maine's lakes, rivers, and streams, and marine and estuarine waters will be monitored for the occurrence of toxic pollutants on an ongoing basis" and that "water, sediment, and biological tissues and aquatic communities will be tested as necessary to indicate exposure to and impacts by toxic contaminants" and "data from the program will be used to support assessment of risks to human and ecological health posed by toxic contaminants."

The results from this study and future work will help enable the Maine Department of Environmental Protection to meet these strategy goals by (1) documenting the extent of Hg contamination to the state's lakes and downstream rivers, (2) using as evidence for ecological damage to public resources and for setting appropriate science-based policy, (3) providing regional and national policy makers with science-based reasons for restricting Hg emissions, and (4) serving as a reference for detecting future changes in atmospheric Hg deposition. We recommend further studies to continue using the Common Loon as the primary ecological indicator of aquatic integrity for multiple geographic and ecological scales in Maine. Specific task for 2001 include:

1) Continue the population-level analysis of Hg impacts on various reproductive measures by targeting 30 territories of each risk category on both reservoirs and natural lakes where their nesting success is not substantially impacted by water level fluctuations and human disturbance.
2) Conduct a sampling strategy that is probability-based across Maine; preferable using the currently established REMAP lake list.
3) Investigate the potentially high MeHg bioavailability for lakes in the Allagash area; preferable based on a current 5-year project for the USFWS and New England Forestry Foundation.
4) Construct a stressor-risk matrix in a spatially explicit way that is based on genetic distinction of subpopulations.

Lastly, because of the high Hg related risk to the Common Loon, we also now recommend a broader sweep of sampling for other species. A flowchart characterizing the extent of risk and future needs follows (Figure 30). Recently discovered "ecological hotspots" such as mountains, reservoirs originating from river valleys, and urban estuaries likely endanger other piscivorous and potentially insectivorous wildlife. We therefore conclude that we should:

1) Assess the impact of Hg on a suite of target species using probability-based sampling. Species choice will be based on trophic levels, habitat use, breeding range, and economic importance, and;
2) Establish ecological references or benchmarks for current and future investigations related to FERC relicensing, NRDA cases, and other mandated policies.

Figure 30 . Flowchart outlining the risk of Hg to Maine's wildlife.

Assessing the Risk of Mercury to Maine's Wildlife: An iterative, comprehensive & integrated approach

PROBLEM STATEMENT: Maine's Common Loon Hg levels are highest in nation

RISK CHARACTERIZATION

EXPOSURE ASSESSMENT

HAZARD ASSESSMENT

STUDIES (Exposure assessment)

- A comparison of Hg exposure and risk between reservoirs and natural lakes in loons and their prey *(FPL,USFWS)*
- Determining Hg exposure in Maine's fish-eating birds *(MDEP, USFWS)*
- Using the Belted King-fisher as a ubiquitous indi-cator across all aquatic habitats *(MDEP)*

STUDIES (Hazard assessment)

- Assessing the impacts of MeHg on piscivorous wildlife as indicated by the Common Loon *(MDEP, USFWS, USEPA)*

RESULTS

1. Hydrology significantly influences MeHg productivity and availability. Reservoirs with a river origin that have summer fluctuations over 1.5 m have greatest Hg risk.

2. Around 70% of Maine's lakes have the ability to produce MeHg levels to create risks to piscivorous wildlife. Although hydrology and biogeographic factors control MeHg availability, mountain and coastal areas are most at risk.

3. When measuring MeHg availability using a standard species across all aquatic habitats, upper watershed lakes and riverine-estuarine habitats were respectively 6x and 3x higher than marine environments.

Weight of evidence indicates 28% of the breeding adult loon population is at risk to impacts from MeHg ingestion.

Parameters showing relationship with high Hg levels:
1. >50% lower hatch/fledge rates
2. 40% of eggs over threshold.
3. Adult/chick behavior lethargic
4. Adults not incubating properly
5. Sig. increase in chick Hg
6. Higher corticosterone
7. Higher asymmetry

WHAT'S NEXT

1. Sample obligate freshwater piscivores
2. Sample high priority insectivores
3. Sample high priority marine piscivore

WHERE

1. Sample on, above, and below reservoirs
2. Sample montane habitat
3. Sample urban estuaries

PRODUCTS

1. GIS-based report with species' reviews available to public and agencies
2. Inventory database available to agencies for FERC, NRDA, etc.
3. Archived samples for benchmarks and future issues that are site-specific / other contaminants.

Acknowledgements

Many individuals, organizations and agencies assisted with this study. BRI field staff included the coauthors and Rebecca Kurtz, Brian Olsen, Dave Yates, and Elayna Zachko. Bill Hanson of FPL Energy Maine Hydro was instrumental for funding certain aspects that contributed to this report and he provided energy and insight into various logistical components of field data collection. We would like to also thank Kate Taylor and her Loon Preservation Committee biologists for assistance with several aspects of the project. Drew Major, Ken Munney and Tim Cozine of the USFWS and Barry Mower of the Maine DEP provided crucial financial and logistical support.

Mark Pokras, Mike Romero, and Rose Miconi provided oversight for the hormone assays. Bob Poppenga of University of Pennsylvania supervised lab analysis for mercury in blood and feathers, while Bob Taylor of Texas A&M's Trace Element Research Laboratory analyzed egg mercury levels. Financial subsidies from both labs greatly assisted in increasing tissue sample sizes. We also thank the Marshall Swain family in Rangeley for the use of boats and assistance in capture efforts. We are grateful to Bob Cercena for providing housing for staff and countless pieces of support and to the Rangeley Lakes Heritage Trust for the use of boats and safety equipment. Numerous thanks to Charlie Adkins and Carolyn Nobbs for housing, advice and the comfort of knowing a "local?. Thanks to this year and past years' crews at Bosebuck Camps who were always there whenever we needed them. We are very grateful to Old Town Canoes and Hamlin Marine for much appreciated discounts on quality equipment and advice.

This study was integrated into the workscope of the Northeast Loon Study Working Group (NELSWG), a coalition of state and federal agency representatives, universities, non-profit organizations and other interested parties and members. We thank all members of NELSWG who contributed their expertise and enthusiasm to this study.

LITERATURE CITED

Altmann, J. 1974. Observational study of behavior: sampling methods. Behavior. 49, 227-267.

Astheimer, L. B., W. A. Buttemer, and J. C. Wingfield. 1992. Intercations of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scandinanica 23:355-365.

Barr, J. F. 1986. Population dynamics of the common loon (*Gavia immer*) associated with mercury-contaminated waters in northwestern Ontario. Occ. Paper 56, Canadian Wildl. Serv., Ottawa, ON, Canada.

Barr, J. F. 1996. Aspects of common loon (*Gavia immer*) feeding biology on its breeding ground. Hydrobiologia 321:119-144.

Benson, K. Baer, and C. Watson. 1990. Metallonthionein as a biomarker of environmental metal contamination: Species-dependent effects. In: J. F. McCarthy, L.R. Shugart (Ed.), Biomarkers of Environmental Contamination (pp. 255-265). London: Lewis Publishers.

Borg, K., H. Wanntorp, K. Erne, and E. Hanko. 1969. Alkyl mercury poisoning in Swedish wildlife. Viltrevy 6, 301-379.

Bouton, S.N., P. C. Frederick, M. G. Spalding, and H. McGill. 1999. Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile Great Egrets. Environ. Toxicol. Chem. 18(9): 1934-1939.

Bradley, D. W. 1985. The effects of visibility bias on time-activity budget estimate of niche breadth and overlap. Auk 102, 493-499.

Burbacher T.M., G.P. Sackett, and N.K. Mottet. 1990. Methylmercury effects on the social behavior of *Macaca fascicularis* infants. Neurotoxicol Teratol 12: 65-71.

Burger, J. 1993. Metals in Avian Feathers: Bioindicators of Environmental Pollution. Reviews in Environmental Toxicology 5, 203-311.

Burger, J., J. Rodgers and M. Gochfeld. 1993. Heavy metal and selenium levels in endangered Wood Storks (*Mycteria americana*) from nesting colonies in Florida and Costa Rica. Archives of Environmental Contamination and Toxicology 24, 417-420.

Burgess, N. M., D. C. Evers, J. D. Kaplan, M. Duggan, and J. J. Kerekes. 1998. Mercury and reproductive success of Common Loons breeding in the Maritimes. Pp. 104-109 *in* Mercury in Atlantic Canada: A progress report. Environment Canada-Atlantic Region, Sackville, New Brunswick.

Caswell, H. 2001. Matrix population models: Construction, analysis, and interpretation. Sinauer Assoc., Inc., Sunderland, Mass.

Clarke, G. 1995. Relationships between developmental stability and fitness: Application for conservation biology. Conservation Biology 9:18-24.

Colborn, F. vom Saal and A.M. Soto. 1993. Developmental effects of endrocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives , 378-384.

Counard, C.J. 2001. Mercury exposure and effects on Common Loon (*Gavia immer*) behavior in the Upper Midwestern United States. Unpubl. MS thesis, University of Minnesota, St. Paul, MN.

Dhar, A. K., M. A. Pokras, D. K. Garcia, D. C. Evers, Z. J. Gordon, and A. Alcivar-Warren.1997. Analysis of genetic diversity in common loon Gavia immer using RAPD and mitochondrial RFLP techniques. Molecular Ecology 1997(6):581-586.

Doving, K.B. 1991. Assessment of animal behavior as a method to indicate environmental toxicity. Comparative Biochemistry and Physiology 100:247-252.

Evers, D. C. 1994. Activity budgets of marked common loon (*Gavia immer*) nesting population. Hydrobiologia 270-280, 415-420.

Evers, D. C. 2001. Common Loon population studies: Continental mercury patterns and breeding territory philopatry. Ph.D. dissertation, Univ. Minn., St. Paul.

Evers, D. C. and P. S. Reaman. 1998. A comparison of mercury exposure between artificial impoundments and natural lakes measured in common loon and their prey. Unpubl. Rept., submitted to Central Maine Power Co. by BioDiversity Research Institute, Freeport, Maine. 40pp.

Evers, D. C., P. S. Reaman, J. D. Kaplan, and J. D. Paruk. 1996. North American loon biomonitoring program: 1989-95 comprehensive report. Unpubl. Rept., BioDiversity Research Institute, Freeport, Maine. 114pp.

Evers, D. C., J. D. Kaplan, M. W. Meyer, P. S. Reaman, W. E. Braselton, A. Major, N. Burgess, and A. M. Scheuhammer. 1998a. A geographic trend in mercury measured in common loon feather and blood. Environ. Toxicol. Chem. 17(2):173-183.

Evers, D. C., P. Reaman, C. De Sorbo, and P. Phifer. 1998b. Assessing the impacts of methylmercury on piscivorous wildlife as indicated by the Common Loon. Unpubl. Rept., submitted to Maine Dept. Environ. Protection, Augusta, Maine.

Evers, D.C., C. DeSorbo, and L. Savoy. 1999. Assessing the impacts of methylmercury on piscivorous wildlifeas indicated by the Common Loon. Unpubl. Rept., submitted to Maine Dept. Environ. Protection, Augusta, Maine.

Evers, J.D. Kaplan, P.S. Reaman, J.D. Paruk, P. Phifer. 2000. A demographic characterization of the common loon in the upper great lakes. Pp. 78-90 *in* J. McIntyre and D.C. Evers (eds.). Loons: Old history and new findings. Proceedings of a Symposium from the 1997 meeting, American Ornithologists' Union. N. Am. Loon Fund, Holderness, NH.

Fimreite, N., W.N. Holsworth, J.A. Keith, P.A. Pearce, and I.M. Gruchy. (1971). Mercury in fish and fish-eating birds near sites of industrial contamination in Canada. The Canadian Field-Naturalist 85, 211-220.

Fox, D.A. and A.J. Silman. 1979. Heavy metals affect rod, but not cone, photoreceptors. Sci. 206: 78-80.

Frederick, M.G. Spalding, M.S. Sepulveda, G. Williams, S. Bouton, H. Lynch, J. Arrecis, S. Leorzel, and D. Hoffman. 1997. Effects of environmental mercury exposure on reproduction, health, and survival of wading birds in the Florida Everglades. Final Rept. Submitted to the Florida Dept. Environ. Protection, Tallahassee, FL.

Friedmann, A. S., M. C. Watzin, T. Brinck-Johnsen, and J. C. Leiter. 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquatic Toxicology 35:265-278.

Gilbertson, M. 1974. Pollutants in breeding herring gulls in the lower great lakes. Canadian Field-Naturalist 88, 273-280.

Gitter, S., A. Pardo, N. Kariv, and U. Yinn. 1988. Enhanced electroretinogram in cats induced by exposure to mercury acetate. Toxicology 51: 67-76.

Gostomski, T. J., D. Evers. 1998. Time-activity budget for common loons, *Gavia immer*, nesting on Lake Superior. Canadian Field Naturalist 112(2), 191-197.

Heinz, G. H. 1979. Methylmercury: reproductive and behavioral effects on three generations of Mallard ducks. Journal of Wildlife Management 43(2), 394-401.

Heinz, G.H. 1996. Mercury poisoning in wildlife. Pp. 118-127 in Fairbrother, A., L.N. Locke, and G.L. Hoff (eds.). Noninfectious Diseases of Wildlife. Second ed. Iowa State University Press, Ames, IA.

Hoffman, D. J., and G.H. Heinz. 1998. Effects of mercury and selenium on glutathinone metabolism and oxidative stress in mallard ducks. Environmental Toxicology and Chemistry 17(2), 161-166.

Hontela, A., Rasmussen, J.B., Audet, C. and G. Chevalier. 1992. Impaired cortisol stress response in fish from environments polluted by PAH's, PCB's, and mercury. Arch. Environ. Contam. Toxicol., 22(3):278-283.

Hontela, A., Dumont, P., Duclos, D., and D. Fortin. 1995. Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence river. Environ. Toxicol. Chem., 14(4):725-731.

Inouye M., K. Murao, and Y. Kajiwara. 1985. Behavioral and neuropathological effects of prenatal methylmercury exposure in mice. Neurotoxicol Teratol 7: 227-232.

Kambamandi-Dimou, A., A. Kamarianos, and S. Kilikidis. 1991. Transfer of methylmercury to hens' eggs after oral administration. Bulletin of Environmental Contamination and Toxicology 46, 128-133.

Kania, H. J. and J. O'Hara. 1974. Behavioral alterations in a simple predator-prey system due to sublethal exposure to mercury. Tran. Am. Fish. Soc. 103:134-136.

LWRC. 1998. Annual Report Appendix A: Initial evaluation and recommendations on Mercury in Maine. Land and Water Resources Council, Augusta, Maine.

Mager, J. 1995. A comparison of the time-activity budgets of breeding male and female Common Loons (*Gavia immer*). M.S. Thesis, Miami Univ., Oxford, Ohio.

Mager, J. 1999. Influence of brood size and chick age on parental effort in common loons. in J. McIntyre and D.C. Evers (eds.). Loons: Old history and new findings. Proc. N. Am. Loon Fund, Meridith, NH.

McIntyre, J.W. 1988. The Common Loon: Spirit of the northern lakes. University of Minnesota Press, Minneapolis. 223 pp.

Marks, G.S. 1985. Exposure to toxic agents: the heme biosynthetic pathway and hemoproteins as indicator. CRC Critical Review in Toxicology 15 (2): 151.

Martin, P. and P. Bateson. 1993. Measuring behavior: an introductory guide. Cambridge: Cambridge University Press.

Meyer, M. W. 1998. Ecological risk of mercury in the environment: the inadequacy of "the best available science". Environmental Toxicology and Chemistry 17(2), 137-138.

Meyer, M. W., D. C. Evers, J. J. Hartigan, and P. S. Rasmussen. 1998. Patterns of common loon (*Gavia immer*) mercury exposure, reproduction, and survival in Wisconsin, USA. Environ. Toxicol. Chem. 17(2):184-190.

Moller, A. and J. Swaddle. 1997. Asymmetry, Developmental Stability, and Evolution. Oxford: Oxford University Press. 298pp.

Mower, B., Jeanne DiFranco, Linda Bacon, and David Courtemanch. 1997. Fish tissue contamination in Maine lakes. Maine Department of Environmental Protection.

Murphy, M. 1996. Energetics and nutrition of molt. In: C. Carey (Ed.), Avian energetics and nutritional ecology (pp. 158-198). New York: Chapman and Hall.

NESCAUM. 1998. Northeast states and eastern Canadian provinces mercury study . NESCAUM, NEWMOA, NEIWPCC, EMAN

Nocera, J and P. Taylor. 1998. *In situ* behavioral response of common loons associated with elevated mercury exposure. Conservation Ecology 2(2), 10.

Olsen, B., D. Evers, and C. DeSorbo. 1999. The effect of methylated mercury on the diving frequency of the Common Loon (*Gavia immer*). Unpubl. Rept.

Ososkov, I. and J. Weis. 1996. Development of social behavior in larval mummichogs after embryonic exposure to methylmercury. Trans. Am. Fish. Soc. 125:983-987.

Paruk, J.D., D. Seanfield and T. Mack. 1999. Bald Eagle predation on Common Loon chick. Wilson Bulletin 111(1), 115-116.

Paruk, J. 1999. Incubating roles and patterns in Common Loons. *In* J. McIntyre and D.C. Evers (eds.). Loons: Old history and new findings. Proc. N. Am. Loon Fund, Meridith, NH.

Peakall, D. P. 1992. Animal biomarkers as pollution indicators. Chapman & Hall, New York.

Piper, W., J.D. Paruk, D.C. Evers, M.W. Meyer, K.B. Tischler, M. Klich, and J.J. Hartigan. 1997a. Genetic monogamy in common loon. Behavioral Ecology Sociobiology 41, 25-31.

Piper, W., J.D. Paruk, D.C. Evers, M.W. Meyer, K.B. Tishler, M. Klich and J.J. Hartigan. 1997b. Local movements of color-marked common loons. Journal of Wildlife Management 61(4), 1253-1261.

Polak, M. and R. Trivers. 1994. The science of symmetry in biology. Trends Ecological Evolution 9, 122-124.

Rahn, H., C. V. Paganellis, and A. Ar. 1975. Relation of avian egg weight to body weight. Auk 92:750-765.

Rumbold, D., P. Rawlik, and L. Fink. 1999. Ecological risk assessment of mercury in the Florida Everglades: Risk to wading birds. South Florida Water Manage. Unit-Mercury/Toxics Unit, Unpubl. Rept.

Sample, B. E. and G. W. Suter, II. 1999. Ecological risk assessment in large river-reservior: 4. Piscivorous wildlife. Environ. Tox. Chem. 18:610-620.

Scheuhammer, A. M. 1991. Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. Environmental Pollution 71, 329-375.

Scheuhammer, A. M., Allan H.K. Wong and Della Bond. 1998a. Mercury and selenium accumulation in Common Loons (*Gavia immer*) and Common Mergansers (Mergus merganser) from eastern Canada. Environmental Toxicology and Chemistry 17(No. 2), 197-201.

Scheuhammer, A. M., C.M. Atchison, A.H.K. Wong, and D.C. Evers. 1998b. Mercury exposure in breeding Common Loons (*Gavia immer*) in central Ontario, Canada. Environmental Toxicology and Chemistry 17(2), 191-196.

Schwartz, J. 1998. The effects of mercury on the swimming behavior of non-larval yellow perch, *Perca flavescens*, in southeastern, New Hampshire. M.S. Thesis, Antioch University, Antioch, New Hampshire.

Smith, G. T., J. C. Wingfield, and R. R. Veit. 1994. Adrenocortical response to stress in the Common Diving Petrel, Pelecanoides urinatrix. Physiological Zoology 67:526-537.

Spann, J., R.G. Heath, J.F. Kreitzer and L.N. Locke. 1972. Ethyl mercury p-toluene sulfonanilide: lethal and reproductive effects on pheasants. Science 175, 328-331.

Stafford, C. P., and Terry A. Haines. 1997. Mercury concentrations in Maine sport fishes. Transactions of the American Fisheries Society 126, 144-152.

Stickel, L. F., S. N. Wiemeyer, and L. J. Blus. 1973. Pesticide residues in eggs of wild birds: Adjustment for loss of moisture and lipid. Bull. Environ. Contam. Tox. 9:193-196.

Sturkie, P. D. (ed.). 1986. Avain physiology. Springer-Verlag, NY.

SWAT. 1997. 1995 Surface Water Ambient Toxic Monitoring Program. Maine Dept. Environ. Protection, Augusta, Maine.

Tacha, T.C., P.A. Vohs and G.C. Iverson. 1985. A comparison of interval and continuous sampling methods for behavioral observations. Journal of field Ornithology 56, 258-264.

Taylor, K and H. Vogel. 2000. New Hampshire Report. Pp. 110-113 *in* J. McIntyre and D.C. Evers (eds.). Loons: Old history and new findings. Proceedings of a Symposium from the 1997 meeting, American Ornithologists' Union. N. Am. Loon Fund, Holderness, NH.

Thompson, D. R. 1996. Mercury in Birds and Terrestrial Animals. In: W. N. Beyer, Gary H. Heinz, Amy W. Redmon-Norwood (Ed.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations (pp. 341-355). Clemson, SC: Lewis Publisher.

USEPA. 1997. Mercury study report to Congress. Volume VII: Characterization of human health and wildlife risks from mercury exposure in the United States. EPA-452/R-97-009.

Weis, J. and P. Weis. 1995. Swimming performance and predator avoidance by mummichog (*Fundulus heteroclitus*) larvae after embryonic or larval exposure to methylmercury. Can. J. Fish. Aquat. Sci. 52:2168-2173.

Welch, L. J. 1994. Contaminant burdens and reproductive rates of Bald Eagles breeding in Maine. Masters of Science, University of Maine: Orono, ME, pp. 87.

Whittier, T. R., David B. Halliwell, and Steven G. Paulsen. 1997. Cyprinid distributions in northeast USA lakes: evidence of regional-scale minnow biodiversity losses. Canadian Journal of Fisheries and Aquatic Sciences 54, 1593-1607.

Wolfe, M. F., Steven Schwarzbach, and Rini A. Sulaiman. 1998. Effects of mercury on wildlife: a comprehensive review. Environmental Toxicology and Chemistry 17(2), 146-160.

Wolfe, M. and D. Norman. 1998. Effects of waterborne mercury on terrestrial wildlife at Clear Lake: evaluation and testing of a predictive model. Environmental Toxicology and Chemistry 17(2), 214-227.

Yablokov, A. V. 1986. Population Biology: Progress and Problems of Studies on Natural Populations . Moscow: Mir.

Yeardley, R. B., Jr., J. M. Laxorchak and S. G. Paulsen. 1998. Elemental fish tissue contamination in northeastern U.S. Lakes: evaluation of an approach to regional assessment. Environmental Toxicology and Chemistry 17(9), 1875-1884.

Appendix 1

BEHAVIOR STATE AND EVENT PROTOCOL

Guidelines for Identifying Behavioral States and Events

Changes from one state to another only occur after 20 seconds into the new behavior with the exception of **Foraging**, in which 2 minutes must pass before recording new state (see exception below). Record the minute and second that each state begins and ends. Events are primarily behaviors that last less than 5 seconds and are not timed. They are tallied (counted) on the data form during the different behavioral states. When recording behaviors in loon territories chick behaviors (states and events) always take priority over adults. States (*S*) and Events (*E*) are indicated next to the behaviors below. In some cases, a behavior can be both a state and event depending on its duration (< or > than 20 seconds). This protocol was originally compiled by Cory Counard and augmented by BRI staff, in order to achieve consistency with those used by Nocera and Taylor (1998).

ADULT–SPECIFIC BEHAVIORS

Courtship: *S* - Formalized and synchronous bill-dipping
Nest Building: *S* - Collection and placement of nest material
Nest Sitting: *S* - Anytime an individual is on nest incubating eggs. Use the following subcategories to characterize this behavioral state:
- **Content:** *S* - In a normal, low energy behavior
- **Alert Posture**: *S* - Neck extended, usually in response to an intruder
- **Sprawl:** *S* - Low profile not in response to intruders but usually due to heat.
- **Hunkered:** *S* - Low profile in response to intruders.
- **Egg Turning:** *S&E* - Sits up and turns eggs with its bill
- **Panting:** *S&E* - Bill open
- **Tending:** *S&E* - Movement of nest material, separate from egg turning

Territorial Encountering (Agonistic): *S* - Interactions with con or interspecifics within the loon's territory. Characterized by circle swimming, bill dipping, splash diving, alert posture and oaring (see Intrusion form to fill out more information). ENCOUNTERING BEGINS WHEN TERRITORIAL BIRDS ARE WITHIN 25 METERS OF INTRUDER (S).
Penguin Dancing (PD): *E* - Body vertical in water with wings tucked in close to body, bill pointed straight ahead or down. This is a very aggressive behavior and is in response to an intruder.
Rushing (R): *E* - Using wings and feet to move over surface of water
Brooding: *S* - One or both adults closely attending one or both young (within 20 feet). Use the following subcategories to characterize this behavioral state:
- **Back Riding:** *S* - 1 or 2 chicks on the back of the adult (if more than one chick record behavior of each)
- **Under Wing:** *S* - 1 or 2 chicks under the wing of the adult (if more than one chick record behavior of each one)
- **Brood-swim:** *S* - Brooding adult swimming and chick(s) in water
- **Brood-drift:** *S* - Brooding adult drifting and chick(s) in water
- **Brood-preen:** *S* - Brooding adult preening and chick(s) in water

Note: note time you suspect subcategory state changed during brooding then write down the time when confirmed (20 second rule applies).

Chick Feeding (CF): *S&E* - Adult(s) present food items to chick(s)

ADULT AND CHICK–SPECIFIC BEHAVIORS

Foraging: *S* - Under water feeding (usually <45 seconds) and above water peering and swallowing. Foraging continues for 120 seconds (2 minutes) after the time of the last diving event, THE EXCEPTION IS PREENING FOLLOWING FORAGING, IN WHICH THE 20 SECOND RULE APPLIES

Locomotion: *S* - Expenditure of energy while swimming above and below water. Underwater locomotion only occurs in response to an intruder. Loons may not be moving when they are swimming against the current or into the wind.

Drifting: *S* - No expenditure of energy while at the water surface. Loons may be moving with a current or wind energy with no expenditure of energy. **Sleeping**-head tuck.

Preening: *S&E* - Maintenance of feathers by spreading oil from the uropygial gland.

Bathing: *S* - Extreme preening behaviors that includes erratic behavior such as flipping upside down.

Foot Waggle (FW): *E* - Anytime foot is above water and shaken. Do not record foot extension while preening or during head scratching.

Head Scratch (HSc): *E* - Foot used for scratching head (**do not record while preening**).

Wing Flap (WF): *E* - Body raised and wings outstretched

Alert Posture (AP): Sitting high in water with neck stretched up / held high

Splash Dive (SD): *E* - Strong kick upward while diving causing water to splash, usually in response to an intrusion.

Peering (P): *E* - Head in water up to or over the eyes with body on the surface. This is different than bill-dipping.

Head Rub (HR): *E* - Roll top of head along (**do not record while preening**)

Vocalizing (V): *E* - Record calls, usually wail, tremolo, hoot, yodel, and mew but can be a combination of all the above.

Yawning (Y): *E* - Raising and extending neck leading to opening and closing of bill as it points skyward.

Bill Dipping (BD): *E* - Rapid immersion of the bill tip into the water followed by a rapid flick of the bill to one side (**do not record while preening**)

Head Shake (HS): *E* - Movement of head back and forth at least twice. Note if this is in response to insects.

Panting (PA): *E* - Adult with bill open and panting bout lasts less than 5 seconds.

Resting (S): - Resting is used in behavior analyses as the sum of drift and sleeping.

CHICK-SPECIFIC BEHAVIORS

In two chick families the priority is to record the behavior of each chick seperately, if this becomes difficult (for example they are both foraging) drop the events on one or both individuals

Underwing: *S* - Chick is under the wing of one of its parents (record if the body of the chick is under the wing)

On Back: *S* - Chick is on the back of the adult.

- **I** = *E* - independent boarding - record if chick gets on the parent without help
- **A** = *E* - assisted boarding - record if parent lifts wing to help chick on the back

Begging (B): *S&E* - Chick swimming close to adult, wanting food.

Fighting: *S&E* - Chicks are fighting (pecking, rushing, etc.); they must both be in the water

Stashed: *S* - Chicks are left near shore while parents are engaging a intruder or threat.

Stashed out of sight: *S* - record time you cannot see chicks

Stashed in sight: *S* - record time you can observe chick (behavior is recorded under Stashed state)